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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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BIOLOGICAL BULLETIN

THE CORRELATION BETWEEN THE CYCLIC CHANGES IN THE UTERUS AND THE OVARIES IN THE GUINEA-PIG.[1]

LEO LOEB.

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In former papers we analyzed the production of the maternal placenta and of the experimental placenta; we followed the cyclic changes in the mammalian ovaries and partly analyzed the factors determining the first step in these changes, namely ovulation. In this paper we shall describe more in detail the cyclic changes in the uterine mucosa of the guinea-pig and correlate these changes with those in the ovaries. We shall analyze the effect of pregnancy, of the corpora lutea and other parts of the ovaries on those changes.

I. THE CYCLIC CHANGES IN THE UTERUS OF GUINEA-PIGS IN WHICH COPULATION TAKES PLACE.

At the period of heat and soon after copulation.—In the uterus the surface epithelium and the gland ducts show high cylindrical epithelium and there can be seen several rows of surface epithelium. The gland fundi have much lower and smaller cells. The number of mitoses in these cells varies. They may be absent or almost absent. Often there are mitoses present in the gland ducts and sometimes in the surface epithelium. They are always absent in the gland fundi. The connective tissue under the epithelium is rather cellular and fibrils may be missing, or be little pronounced; in the deeper connective tissue they are more prominent. Mitoses are usually lacking in the connective tissue, but a few may be present especially near the epithelium of the surface of the gland ducts. Some mononuclear cells and also a few polynuclear leucocytes can be seen in the mucosa and may migrate through the surface epithelium, or collect in its neighborhood. The uterus is usually slightly papillary. There is no marked hyperemia in the mucosa, which, however, is somewhat edematous. Some gland fundi are cystically dilated, and partly filled with leucocytes, partly without leucocytes.

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Within the first hour after copulation the uterine epithelium (of the surface as well as of the gland ducts) is high cylindrical; in the gland fundi we find lower cuboidal cells; inasmuch as the quantity of cytoplasm is relatively small in the gland fundi, the blue color predominates if the sections are stained with haematoxylin and eosin. The glands as a whole are small, straight and widely separated. Mitoses are more or less frequent in the surface epithelium and in that part of the glands in which the cells are high cylindrical, the mitoses are, however, more frequent in the glands. The connective tissue is rich in cells, the nuclei are somewhat larger, the tissue is in parts somewhat edematous, especially towards the surface; no mitoses are seen in the connective tissue. Some gland ducts are filled with polynuclear leucocytes; these are also present around glands; many glands, however, are free from polynuclears. Small mononuclear cells migrate through the surface epithelium. One hour after copulation the condition of the mucosa is almost the same as five minutes after copulation; the number of the mitosse in the surface epithelium has perhaps been somewhat decreased. The mucosa is quite edematous; a mitosis is seen in the connective tissue (perhaps in an endothelial cell.) Some spermatozoa are visible in the lumen of the uterus and of the glands. Leucocytes migrate through the mucosa into the glands.

Three, four and five hours after copulation, the surface epithelium is high cylindrical. Polynuclear leucocytes migrate through the surface epithelium and may injure epithelial cells of the surface epithelium; vacuoles may appear in the surface epithelium. As the result of the injurious effects of the leucocytes, the surface epithelial cells may become lower and their nuclei deformed and contracted. There are not any, or only a few mitoses in the surface epithelium. The gland ducts are high cylindrical; they appear with hæmatoxylin-eosin red-stained, the cytoplasm preponderating. The ducts are short, the gland fundi have lower cuboidal epithelium. In the gland ducts there are some mitoses present; they are missing in the gland fundi where the cells are cuboidal. Polynuclear leucocytes migrate also through gland epithelium into the glands and may occasionally injure the gland epithelium. The connective tissue shows a distinct fibrillar character; its nuclei are rather large; it is somewhat edematous; some leucocytes migrate from the vessels through the connective tissue towards the epithelium. There are a few mitoses in endothelial cells of blood vessels and possibly also in connective tissue cells. The uterus shows blunt papillae. The sperm fluid present in the lumen of the uterus exerts a pressure on the surface epithelium and may thus contribute to the harmful influence of the leucocytes. In a specimen eight hours after copulation, the same results were obtained. There were, however, numerous mitoses in the surface epithelium.

It seems, therefore, as if the number of mitoses in the surface epithelium a few hours after copulation was determined by accidental conditions such as the action of leucocytes and pressure of the fluid, and was, therefore, variable to the same extent as these factors varied. The leucocytes may pass in such quantities through the mucosa into the lumen of the uterus that the fluid in the uterine cavity appears like an abscess and the leucocytes almost destroy the surface epithelium at certain places. Agglutinated spermatozoa are seen in the lumen of the uterus a few hours after copulation, and spermatozoa are also seen in the gland ducts. The polynuclear leucocytes migrate in large numbers through the connective tissue of the mucosa, through the epithelium and the gland ducts. Under their influence much of the surface epithelium has been destroyed and even the epithelium of the gland ducts has been loosened.

Soon after parturition the guinea-pig is ready to copulate. At this time the mucosa is papillary and low. The muscle tissue lies at a number of places near the surface epithelium; the glands are therefore short; the epithelium is cuboidal or cylindrical, at some places high and with much cytoplasm; the vesicular nuclei are often at the base of the cell. Sometimes there are vacuoles in the epithelial cells. Mitoses are extremely rare in the epithelium; the gland cells are small and without mitoses. The connective tissue in the mucosa is somewhat edematous; it forms only a small layer; the nuclei are not large, rather densely packed; mitoses are not visible. At some places the epithelium is absent and coagulated blood with polynuclear leucocytes covers the surface. The muscle tissue is turgid and some plasmodia are visible around the vessels of the muscular coat. The condition of the surface epithelium is here apparently similar to the epithelium during the period of heat.

From twelve to twenty-four hours after copulation.—This period is marked through the great number of polynuclear leucocytes seen in the lumen of the uterus, in the lumen of the glands and especially in the upper ducts of the glands. There may be also present abscess-like collections of polynuclear leucocytes in the connective tissue of the mucosa. They may raise up the surface epithelium from the underlying connective tissue at some places; at other places they rarify and almost destroy the epithelial cells. We may still see twelve hours after copulation the high cylindrical shape of these cells. The glands are short and they do not show much branching; the gland ducts still show a lining of cylindrical cells; some gland-lumina are dilated through masses of leucocytes and the leucocytes may exert such a pressure on the walls of the glands that the epithelium becomes flat. Mitoses are seen at this period.

From one to four days after copulation.—Two days, five and a half hours, and two days, seven hours after copulation, the epithelium of the surface and of the gland ducts is cylindrical and usually high; the gland fundi do not show as high an epithelium. There are mitoses present in the surface epithelium and in the upper and medium parts of the glands; but the mitoses may reach also further into the lower part of the glands. The gland fundi are not large nor separated from each other by much connective tissue. The connective tissue of the mucosa contains many nuclei; the fibrils are not pronounced and the tissue has a succulent, somewhat myxoid appearance; the nuclei are vesicular. Between the gland fundi the connective tissue is more fibrillar. No mitoses are as yet visible in the connective tissue of the mucosa; some leucocytes are still to be found there. We see, therefore, that the large masses of leucocytes have mostly disappeared as early as two days after copulation, and that the damage done to the epithelial structure has been repaired.

In the first half of the fourth day after copulation the epithelial structures are on the whole unchanged; the surface and gland duct epithelium is cylindrical, but at the surface there may be some cuboidal cells; the gland fundi are still composed of smaller cells, but in the second half of the day, the gland fundi also become active and show frequent mitoses. In the surface epithelium there are at this period either none at all, or only exceptional mitoses. At this period begins also the increase in the number of connective tissue cells in the mucosa. Three days, eight hours after copulation, the connective tissue of the mucosa has a myxoid character; the nuclei of the connective tissue cells are still small, but a little larger than at former periods; only exceptionally a mitosis is visible in the connective tissue of the mucosa. Many blood vessels are present and the tissue appears hyperemic, but no mitoses are seen in the vessels. The connective tissue between the fundi is denser. Three days and twelve hours after copulation, the mucosa is wider; there are round or oval vesicular nuclei present in the connective tissue cells; their cytoplasm is permeated with vacuoles; a certain though not yet large number of mitoses is seen in the connective tissue cells and these mitoses are independent of mitoses in endothelial cells of blood vessels which also occur. Thus the connective tissue cells become gradually transformed into predecidual cells. The connective tissue assumes a similar structure as regenerating connective tissue in wounds, the vessels running at right angles to the bundles of connective tissue cells. The enlargment of the connective tissue causes a drawing out of the gland ducts and now mitoses appear also in the gland fundi, perhaps as a result of the pull exerted on the gland ducts. Some leucocytes migrate through the mucosa and epithelium.

About four days after copulation, or soon afterwards, the surface epithelium has become lower; it is either cuboidal or cylindrical, of medium height. No mitoses are present in the surface epithelium or in the cuboidal or low cylindrical gland ducts. The cells in the fundi of the glands are cylindrical and there are very many mitoses present. The mitoses migrate therefore gradually from the surface into the gland fundi, and concomitantly with the decrease in mitoses we find a decrease in the size and shape of the epithelial cells of the surface and gland ducts. In the connective tissue there is now a decided increase in the number of mitoses especially in cells near the surface and gland epithelium. The connective tissue cells themselves have vesicular nuclei and form fibrillar processes. Between the gland fundi the intercellular fibrils are more marked; the number of nuclei is smaller and there are less mitoses present. The tissue may, however, be edematous. The mitoses are mainly present in the fibroblasts, but there are some mitoses also in endothelial cells of capillaries. The proliferation takes place principally in the fibroblasts and the predecidual cells do not originate in the endothelium of the blood vessels. The vessels are often running directly towards the surface epithelium and at right angles to the direction of the connective tissue cells. There are occasionally some polynuclears present in alveoli of glands.

Six to eight days after copulation.—Six days, and on the seventh day after copulation, the surface and the gland epithelium is cuboidal low, or medium cylindrical. The gland ducts are drawn out. In the glands there may still be some higher cylindrical epithelial cells left; nowhere are mitoses present in the epithelium. There are mitoses in the connective tissue underneath the surface epithelium and between the gland ducts. The arrangement is the one typical for regenerating connective tissue, the vessels running at right angles to the layers of the connective tissue. Below the epithelium there are layers of densely packed large vesicular nuclei; the cells are often free, not enveloped by fibrils. The number of mitoses varies somewhat at different places. The gland fundi are surrounded by edematous connective tissue and between the gland fundi we find fibrillar connective tissue. The cellular connective tissue separates the gland fundi from the surface epithelium. Some polynuclear leucocytes are in the mucosa; we see here some disintegrating cells; it is possible that they are mainly polynuclear leucocytes.

Seven days, and on the first half of the eighth day after copulation, the surface epithelium is cuboidal or low-medium cylindrical; at a few places it is somewhat higher with vacuoles in the outer part of the cells; the nuclei are small vesicular. Usually no mitoses are present, but in one piece a few mitoses could be seen.

The epithelium of the glands is cuboidal or low cylindrical; the glands are drawn out. In one piece there are still a number of mitoses in the gland fundi, although the gland cells are here cuboidal. The structure of the connective tissue is similar to that of the previous period. There are mitoses present in the cell layer but they are not quite as frequent as before. The cell layer consists of round, relatively small cells with many capillaries with narrow endothelium. There is some hyperemia present. At other places the nuclei in the cell layer are round vesicular, and the cytoplasm of the neighboring cells is connected by bridges.

Eight days, eight hours, after copulation, the structure of the surface epithelium and glands is similar to the previous period. The epithelial cells are low. No mitoses are present. The glands are not very large and contain a relatively small number of coils. There is still some cell layer present under the surface epithelium; the vesicular nuclei are compressed and a number of nuclei under the epithelium disintegrate, perhaps the nuclei of the proliferated connective tissue layer.

Eight to seventeen days after copulation.—In order to prevent pregnancy the tubes had been ligatured near the junction with the uterus soon after copulation.

Ten to eleven days after copulation.—The surface epithelium is lower than at the beginning of the sexual cycle; it is cuboidal or low cylindrical; at certain places there may be swollen cells. There are generally no vacuoles in the epithelium; mitoses are usually missing; they were present only in one place. The glands are small coiled, lying near the surface, are without a wide lumen, their cells are not high and without mitoses. The connective tissue of the mucosa is well supplied with capillaries, which run at right angles to rows of connective tissue cells. The connective tissue is still partly myxoid under the surface of the epithelium. At some places there are still many vesicular nuclei densely packed. In the deeper connective tissue between the coils of the glands there are smaller nuclei and some fibrils. Only one mitosis is seen in a connective tissue cell in the mucosa. There is some nuclear debris present under the epithelium; some mononuclears are in the mucosa tending to migrate through the epithelium.

Twelve to fourteen days after copulation.—Low cuboidal or low cylindrical epithelium of surface with *frequent* mitoses, especially at places of invagination of the epithelium; glands with low cuboidal or low cylindrical epithelium without mitoses. In the connective tissue there are no mitoses; small vesicular nuclei of connective tissue cells under the epithelium of the surface. The further the connective tissue is removed from the surface epithelium, the more the fibrils become developed and the smaller the nuclei. There is occasionally a little edema in the connective tissue. Some small mononuclear cells may migrate through the epithelium. The layer of proliferated connective tissue cells which was previously present is much smaller now than five to seven days after ovulation. The glands are correspondingly smaller. At this period mitoses appear again in the surface epithelium.

Fifteen to seventeen days after copulation.—The epithelial cells are low cuboidal; mitoses are present only in the surface epithelium. The connective tissue contains small nuclei and is fibrillar. Near the surface epithelium the nuclei are relatively more frequent and vesicular in the connective tissue, and the fibrils are less developed than in the deeper parts of the mucosa.

Occasionally some edema is present in the surface epithelium and at such places vacuoles are present in the epithelial cells.

This condition of the uterus may exist notwithstanding the presence of follicles in the ovaries which are near maturity. The latter do, therefore, at least as long as the corpora lutea functionate, not produce the changes in the mucosa of the uterus which are characteristic of the first period in the sexual cycle.

During pregnancy the uterus below or above the insertion of the placenta or in the horn in which there happens to be no embryo shows low cuboidal or low cylindrical epithelium with or without mitoses. There may be some vacuoles in the epithelial cells. There are small narrow coiled glands with low cylindrical epithelium. There is no large connective tissue cell layer present. The nuclei in the connective tissue of the mucosa are small and surrounded by well developed connective tissue fibrils; especially between the gland fundi do we find large connective tissue fibers, while underneath the epithelium the number of connective tissue nuclei is relatively greater and the tissue is less fibrillar. Mitoses occur neither in the glands nor in the connective tissue. It seems that in the earliest stages of pregnancy less than ten days after copulation, there are not any or only exceptional mitoses in the surface epithelium; correspondingly we saw previously that six to ten days after a previous period of heat—copulation having been prevented—no mitoses were found in the surface epithelium. During the middle or end of pregnancy the uterine mucosa has a similar structure; but there may be present some papillæ in the mucosa. The presence of mature, or nearly mature follicles in the ovaries is without influence on the structure of the uterus during pregnancy.

II. The Sexual Cycle of Uterus and Ovaries in Guinea-Pigs in Which Heat had been Observed but Copulation Prevented, is as Follows:

At the period of heat previous to the rupture of the follicles we find the following condition: In the ovaries we find one or more small corpora lutea which are just beginning to degenerate (vacuolization in the periphery). The follicles with exception of the smallest show degeneration of the granulosa. There may be at earlier stages still some good medium-sized follicles. There are present one or several mature follicles usually with maturation spindle in the ovum of the follicle. Other follicles show early and medium stages of connective tissue atresia. There are usually many follicles in or near the last stage of connective tissue atresia, but the number of such follicles varies and may be relatively small in very young ovaries at the time of the first heat. The structure of the uterus at this period has been described above (see page 1). It is identical with the structure of the uterus in the period of heat when the latter is followed by copulation. Even after extirpation two months previously of the greater part of the uterus, the same phenomena can be observed; heat takes place and mature follicles form. The remaining small part of the uterus forms a little cyst with cylindrical (or cuboidal) epithelium with many mitoses. The gland ducts have highly cylindrical epithelium and also show mitoses. This has been observed in two experiments. Also after almost complete extirpation of the thyroid one to two months previously, heat occurs accompanied by the typical changes in the uterus and ovaries. The small remaining part of the thyroid may consist of acini with cylindrical epithelium while the colloid may be almost, or entirely absent.

At a slightly later period, namely, soon after the rupture of the mature follicles, the condition of the ovaries and uterus is essentially the same; two changes have however, taken place, namely (1) the surface epithelium is not quite so regularly high cylindrical, but may be at certain parts cuboidal; and (2) we find more or less pronounced changes in the surface epithelium and sometimes in the adjoining part of the gland ducts, namely, vacuolization in the epithelial cells; in the vacuoles which press upon the nuclei we may find degenerating cells; chromatin particles may spread over the greater part of the cells. These changes are probably due to the immigration of mononuclear cells or polynuclear leucocytes from the mucosa into the epithelium. Here these immigrated cells degenerate. One sees sometimes mononuclear cells immigrating into the epithelium. It is not very probable that the changes in the epithelium following ovulation are brought about through a disintegration of some epithelial cells.

Mitoses are very rare in the epithelial structures; there may be however a very small number present in the surface epithelium or in the upper gland ducts. Some glands at this period are dilated and filled with polynuclears. Only rarely do we see a mitosis in the connective tissue. We find in some pieces soon after the rupture of the mature follicles hemorrhages beneath the epithelium which may raise the surface layer of the epithelium from the underlying connective tissue.

The same condition we find also in the course of the first day after the rupture of the follicles, at a time, therefore, when connective tissue begins to grow into the follicular cavity. Perhaps the degenerative changes in the surface epithelium are even more marked at this period.

In four animals in which the ovaries and uterus were examined seventeen and eighteen days after a previous heat, we found ruptured follicles in the animals examined (after seventeen days) or very young corpora lutea (after eighteen days) and the corresponding condition of the uterine mucosa.

We may therefore conclude that in animals kept under normal conditions the period of heat occurs approximately every fifteen to nineteen days, a conclusion that is corroborated by other observations which we made. We furthermore conclude that the condition of the uterine surface epithelium indicates in most cases whether or not a rupture of a follicle has taken place in the ovary.

In a specimen obtained *about one to one-and-a-half days after spontaneous ovulation*, in which no copulation had taken place, there were present at most places high cylindrical epithelium of the surface; at some places, however, the epithelium was somewhat lower; gland fundi show as usual lower epithelium. In the surface epithelium and in the gland ducts with higher epithelium there are mitoses present. The mucosa is rich in cells; is partly a little edematous; there is no increase in the size of the mucosa visible and no mitoses are seen. Some polynuclear leucocytes are present in the mucosa, connective tissue as well as glands, although no spermatozoa had entered the uterus; but the number of leucocytes is much smaller than in cases in which copulation had taken place.

Three days after heat we find a vacuolar corpus luteum of the former period, in process of degeneration. In the central cavity of the new corpora lutea new connective tissue is present in the periphery. There are many small, and small to medium-sized good follicles; many follicles in an early-medium stage of connective tissue atresia, and a large number of follicles in the late stages of connective tissue atresia.

The surface epithelium of the uterus is mostly high cylindrical, but may be at places cuboidal, probably as a result of stretching. It does not show any longer the changes observed in the first two days after ovulation. It is usually free from mitoses; the gland duct epithelium is high cylindrical and the epithelium of some fundi begins to become also higher. There are many mitoses present in the gland ducts, especially in their medium parts; the mitoses begin now to proceed further downwards and occasionally may be found in these gland fundi which have enlarged. The connective tissue of the mucosa is, especially in the higher parts, rather cellular, with vesicular nuclei, but without mitoses; it may be somewhat myxoid.

In the mucosa we find some small mononuclear cells and also some polynuclear leucocytes. Some mononuclears may immigrate into the gland and surface epithelium. There may be seen some cystically dilated glands.

Four days after heat the corpus luteum of the former period is vacuolar; the new follicles are well developed. There are many small and medium-sized good follicles, and many follicles in medium or the last stage of connective tissue atresia. The epithelium of the surface and gland ducts is no longer as high as before; it is cylindrical, of medium height, or cuboidal-cylindrical; the epithelium of the gland fundi is higher than before. There are many mitoses present in the gland fundi; but nowhere else in the epithelium. Some glands are dilated and contain polynuclear leucocytes. The connective tissue layer under the epithelium is enlarged and shows frequent mitoses. The uterus is slightly papillomatous and in the connective tissue of the papillæ there is some edema. The epithelium over the edematous connective tissue may also be edematous.

Five days after heat, we find in the ovaries many small and medium-sized good follicles, a number of follicles in a medium stage of connective tissue atresia, and there are a large number of follicles in the last stage of connective tissue atresia. The surface and glandular epithelium is cylindrical, of medium height and is almost without mitoses; a few isolated mitoses may be found in the gland fundi. The cell layer of the connective tissue contains many mitoses; there are some small mononuclear, and a few polynuclear leucocytes in the mucosa.

Six days after heat, the corpora lutea of the former period are quite vacuolar and in process of degeneration. There are good new corpora lutea and many good small, medium-sized and large follicles, and many follicles in the last stages of connective tissue atresia. The surface epithelium of the uterus is cylindrical and of medium height; the epithelium of the gland ducts and gland fundi is cuboidal-cylindrical. There are nowhere mitoses present in the epithelium. There are mitoses present in the connective tissue cell layer of the mucosa, but the mitoses are here probably not as frequent as at five days after the previous period of heat. There are many small mononuclear cells in the connective tissue.

Seven days after heat, we find good corpora lutea, many good small medium-sized and large follicles, no follicles with granulosa degeneration and many follicles in the last stage of connective tissue atresia. The epithelium of the surface and glands of the uterine mucosa is cuboidal or cylindrical of medium height. No mitoses are present in the epithelium; some mononuclears can be found in the surface epithelium. In the connective tissue cell layer there are a number of mitoses, but not as many as five days after heat. Some polynuclear leucocytes and small mononuclear cells are in the mucosa and some nuclei disintegrate in the upper layer of the connective tissue.

Eight days after heat, the condition of the ovaries is the same as seven days after the period of heat, but degeneration of the granulosa has now set in in some of the larger follicles. The corpora lutea of the former period are quite vacuolar and are in full process of retrogression. The epithelium of surface and glands of the uterus is cuboidal or cylindrical of medium height, without mitoses. In the connective tissue of the mucosa we still see blood vessels running towards the surface and at right angles to the long axis of the connective tissue cells; but mitoses are no longer present and some cells disintegrate in the upper part of the mucosa.

Nine days after heat.—There is as yet not any, or very little granulosa degeneration in the large follicles; occasionally there may be more extensive degeneration of the granulosa and a few exceptional follicles may show an early stage of connective tissue atresia; in some ovaries all the large follicles are still well preserved. The follicles which six to eight days after the former period of heat were in the last stage of connective tissue atresia, shrink under the influence of the pressure of the growing follicles and corpora lutea.

The uterine epithelium of the surface is low to medium-sized cylindrical, a few vacuoles may be present in the epithelium, the gland epithelium is low cylindrical. The connective tissue of the mucosa shows still vesicular nuclei and vacuoles in the cells, but it is being somewhat compressed. There are no mitoses present in the epithelium or in the connective tissue.

Eleven days after heat.—We find good small, medium-sized and large follicles, some large follicles show granulosa degeneration or connective tissue in growth. The follicles which were formerly in the last stage of connective tissue atresia begin to disappear more and more; there are still the remains of the membranæ pellucidæ, surrrounded by some very small compressed nuclei. The corpora lutea are in a good condition. Mature follicles appear again in the ovaries.

The epithelium of surface and gland ducts of the uterus is cyclindrical, of medium size. The gland fundi are smaller. No mitoses are seen; some mononuclear cells may be present, but neither vacuoles nor chromatin particles; there is still some remnant of the cell layer in the mucosa, but it is becoming smaller and more fibrillar; only an exceptional mitosis can be found in the connective tissue.

We find, therefore, active cell proliferation in the connective tissue of the mucosa from the fourth to the seventh day after the last ovulation. Approximately five days after ovulation the mitoses disappear also in the gland fundi. From the sixth to the twelfth day after ovulation, the epithelium of the uterine mucosa is without mitoses. Then they begin to reappear.

Twelve days after heat.—There are many good small, medium-sized and large follicles; some large follicles show granulosa degeneration. A number of follicles show early and medium and some advanced connective tissue atresia. The follicles which had arrived about six to seven days after copulation at the last stage of connective tissue atresia are disappearing more and more. Some mature follicles may be present.

The surface and glandular epithelium of the uterus is cuboidal-cylindrical; some small mononuclear cells migrate through the surface epithelium. No definite mitoses can be seen in the surface epithelium. A few isolated gland ducts and the adjoining surface epithelium have high cylindrical epithelium. The mucosa is narrow and the nuclei of the connective tissue are small.

Fourteen days after heat.—In the corpora lutea some vacuolization begins in the periphery. There are good, small medium-sized and large good follicles, some large follicles show granulosa dgeneration; other follicles are in the early or medium or a later stage of connective tissue atresia. These follicles which are now constantly degenerating all contribute to the follicles in the late stages of connective tissue atresia which we find at the time of heat.

The surface and gland duct epithelium of the uterus is cuboidal or cylindrical of medium height. The gland fundi are smaller. There are some mitoses present in the surface and gland duct epithelium. The mucosa is low, the connective tissue nuclei are small. There is some edema present in the connective tissue which may even raise up the surface epithelium. There may be some dilated glands with polynuclear leucocytes and some mononuclears in the mucosa.

Fifteen days after heat.—At this time the ovaries are about the same as fourteen days after heat. There are good small, medium-sized and large follicles; a number of large follicles show granulosa degeneration. A certain number of follicles are in the medium- or medium-late stages of connective tissue atresia. There are not many follicles in the last stage of connective tissue atresia. The follicles which degenerated at the period of the last heat have almost disappeared and the other degenerating follicles have not yet advanced to the last stage of atresia. There are mature follicles present. The periphery of the corpora lutea show some slight vacuolization. The connective tissue of the uterine mucosa is at this period very edematous, the edematous fluid may raise the surface epithelium. The connective tissue nuclei are very small. The mucosa and also the muscularis of the uterus are thin. The epithelium of the surface and of the gland ducts is low cuboidal or low cylindrical. This is perhaps partly due to the pressure of the underlying edematous fluid. Where the connective tissue is very edematous, the overlying epithelial cells may also be somewhat edematous and vacuolar. At some places the epithelium of the surface and gland ducts is somewhat higher. Some mitoses are present in epithelial cells of the surface and at the entrance of the gland ducts. Exceptionally a mitosis may be found in a gland duct.

We see, therefore, that from the fourteenth day on, mitoses reappear in the surface epithelium of the uterine mucosa. If we compare the cyclic changes in the uterus in cases in which copulation took place and in which only the heat was observed, but copulation had been prevented, we find the following:

A few hours after copulation, the leucocytes begin to migrate in large numbers from the connective tissue of the mucosa through the surface epithelium into the lumen of the uterus. Here they collect in such large numbers eight hours after copulation that its contents may be like an abscess.

These leucocytes exert an injurious influence on the surface epithelium which is therefore after copulation very much earlier and very much more strongly injured than if copulation has been prevented. The epithelium of the gland ducts may also suffer. We find, a few hours after copulation, spermatozoa which may be agglutinated in the lumen of the uterus and also in the gland lumen. The same large number of polynuclear leucocytes is still present during the first twenty-four hours after copulation; they have however mostly disappeared two days after copulation. At that time the condition of the uterus is very similar in animals in which copulation took place and in which copulation had been prevented. A few leucocytes can also be seen in the uterus of animals in which copulation had been prevented. In such animals also some degenerative changes occur in the uterine epithelium, but they are less marked than in animals which had copulated. An examination of the uterus permits us in most cases to determine whether or not ovulation had taken place at the time of the examination in a guinea-pig in which copulation had been prevented.

From now on, the condition of the uterus is similar in both series of guinea-pigs; the mitoses migrate from the surface epithelium to the gland fundi and disappear completely in the epithelium five to six days after the ovulation. The connective tissue proliferation starts on the fourth day and ceases seven days after ovulation. On the eighth day we find a beginning disintegration of cells in the upper part of the connective tissue of the mucosa. The later stages were also examined in the first series in animals in which after copulation, pregnancy had been prevented through an early ligature of the tubes. From the sixth to the twelfth day after ovulation the epithelium is without mitoses; they then begin to reappear in the surface epithelium. Notwithstanding the presence of mature follicles, the uterus does not assume the characteristic features of the uterus during heat. These changes occur only after signs of degeneration have begun to set in the corpora lutea.

During pregnancy the uterine epithelium is relatively low, mitoses occur only in the surface epithelium. The connective tissue layer is thin and no mitoses are found in the connective tissue cells. The uterus is therefore in a resting condition during pregnancy.

III. THE EFFECT OF EXTIRPATION OF THE CORPORA LUTEA ON THE CYCLIC CHANGES IN THE UTERUS.

In a previous communication we have shown that extirpation of the corpora lutea within the first week after ovulation leads in the majority of cases to an acceleration of the next ovulation. In nine cases in which pregnancy was not present at the time of the examination, the ovulation had not yet taken place notwithstanding the complete extirpation of the corpora lutea. In four of these cases the uterus had been split within the first week after ovulation. In another case abortion had taken place at the time of the examination; in the sixth case the guinea-pig had been in a very weak condition and in the ovaries no good large or mature follicles, but some large follicles in granulosa degeneration, were found. In the seventh case the corpora lutea had been burnt instead of excised with the scissors. As we saw previously, the burning out of the corpora lutea injures the ovaries and prevents in many cases an early ovulation. In four cases in which the uterus had been cut, and in one other case, one ovary had been excised previously.

The uterus in these cases in which ovulation had not been hastened through extirpation of the corpora lutea was found to be as follows:

(1) No. 642. Three days after copulation extirpate corpora lutea examination. Sixteen days after the previous copulation the animal was in a weak condition. In the ovaries there was no good large or mature follicle, there were some large follicles with granulosa degeneration. The surface epithelium of the uterus was cuboidal; it contained some vacuoles with chromatin particles; there were very few mitoses present. The uterine glands were cuboidal to cylindrical and showed no mitoses. The connective tissue was fibrillar, the nuclei near the surface epithelium were more numerous; no mitoses were present in the connective tissue.

(2) No. 693. Seven days after the previous copulation extirpate the corpora lutea; ten days later examination. Abortion had taken place. The ovaries showed small and large good follicles and large follicles with granulosa degeneration; mature follicles were present. There were also follicles in various stages of connective tissue atresia.

The surface epithelium of the uterus was cuboidal to cylindrical and showed many mitoses; the glands were small and without mitoses; there was fibrillar connective tissue; where abortion had taken place, the connective tissue was hyperæmic, edematous and there were many polynuclears in the vessels.

(4) No. 300. Twenty-three hours after copulation extirpate left ovary; six days later make incision into the uterus; seventeen days after copulation, examine the uterus. In the remaining ovary of the animal which was about four months old, no corpus luteum and only small and small-medium sized good follicles

were found, besides follicles in an early and medium stage of connective tissue atresia. The uterus shows low cylindrical epithelium and glands with similar epithelium; some glands are dilated. There is a very small number of mitoses in the surface epithelium and in the superficial part of the gland ducts; the connective tissue is fibrillar.

The uterus is similar in No. 270 (four days after copulation excise left ovary, three days later cut out the uterus, examination seventeen days after copulation). In the ovary there was no mature follicle. The epithelium of surface and glands of the uterine mucosa was low, mitoses only present in the surface epithelium; the connective tissue of the mucosa was fibrillar and with small nuclei, without mitoses.

Similar were the findings also in No. 93 (three and a half days after copulation extirpate left ovary, one day later make incision in the uterus, examination fourteen and one half days after copulation). In this case there is however at some places somewhat higher surface and gland epithelium. In No. 992 (six days, ten hours after copulation one ovary was extirpated; the uterus was not cut; eighteen days ten hours after the previous ovulation, the examination was made). In this case the uterine epithelium was low cuboidal to cylindrical and contained mitoses; the glands were similar without mitoses, the connective tissue underneath the epithelium had some larger vesicular nuclei, and became fibrillar somewhat deeper.

In No. 843 the corpora lutea had been extirpated six days, sixteen hours after copulation; ovaries and uterus examined twenty and one half days after ovulation; there were many good medium-sized follicles present, others showed granulose degeneration, still other follicles were in various stages of connective tissue atresia. The surface epithelium was cylindrical, of medium size. The gland duct epithelium was similar. There were frequent mitoses present in both; the connective tissue is fibrillar. At one place the uterus had been thickened. Here the connective tissue was somewhat edematous, the connective tissue nuclei were small; there was a mitosis present in a connective tissue cell at this place. In this case abortion had probably taken place at the thickened place. In No. 105 corpora lutea had been burnt three days, twenty hours after copulation; twenty and one half hours later incisions were made into the uterus and fourteen days, seventeen hours after copulation, ovaries and uterus were examined. There were mature follicles present in the otherwise typical ovaries. The uterus was similar as in No. 270. The uterus was also similar in No. 391, where two days, seven hours after ovulation, the right ovary had been excised and one corpus luteum of the left ovary. Five days later the uterus had been incised and seventeen days after the last copulation ovaries and uterus were examined. No mature follicles were found in the ovaries.

In one case (No. 687) the corpora lutea had been extirpated seven days, seven hours after copulation and ten days later the uterus and ovaries were examined. In the uterus two pregnancies were found; the mucosa of the uterus above and below the decidua was poor in nuclei and fibrillar. In the ovaries there were mature follicles, but no new ovulation had as yet taken place.

We may conclude that in cases in which after an early extirpation of the corpora lutea no new ovulation takes place, the condition of the uterus does not undergo any marked changes.

As we stated previously in the majority of cases in which within the first week after ovulation the corpora lutea had been cut out with scissors a new ovulation takes place between the eleventh and sixteenth day after ovulation. If we examine the uterus in these animals we find the typical structure which we might expect in accordance with the time since the last ovulation.

(1) No. 669. Forty-nine hours after last copulation, extirpation of the corpora lutea. Examination of the ovaries and uterus twenty days after the last copulation. Examination of the ovaries revealed that an ovulation had taken place about seven to eight days previously; correspondingly we find in the uterus the typical condition: rather low cylindrical surface epithelium, small glands with the same epithelium, in the connective tissue of the mucosa small vesicular nuclei, pressed together; mitoses are lacking in epithelial as well as connective tissue cells.

(2) No. 772. Two days, seventeen hours after last copulation extirpate corpora lutea; examine uterus and ovaries not quite

eighteen days after copulation. Four to five days before the examination a new ovulation had taken place. The epithelium of the surface was found cylindrical of medium height without mitoses; the glands had the same epithelium; there were mitoses present in the fundi of the glands. The connective tissue of the mucosa showed a layer of vesicular nuclei; it has a somewhat myxoid character and contained many mitoses. This condition of the uterus was in accordance with the time of the last ovulation.

(3) No. 773. Two days, eight hours after copulation, extirpate the corpora lutea; sixteen days later examine uterus and ovaries. Ovulation had taken place about seven days previously. Surface epithelium and glands of uterus show cuboidal or cylindrical epithelium of medium height; there are no mitoses present. In the connective tissue there are still a large number of proliferated cells, but only one mitosis is visible in the connective tissue under the epithelium. This corresponds to the picture of the uterus about seven to eight days after copulation.

(4) No. 779. Six days after copulation extirpate corpora lutea. Twelve days later examine uterus and ovaries. The ovaries show that rupture had taken place about four days previously. Surface epithelium and gland cells are cylindrical, relatively high, there are many mitoses in the gland fundi; they are lacking elsewhere in the epithelium. Small mononuclear cells migrate through the epithelium; the connective tissue cells are swollen and are densest under the surface epithelium; they form a distinct cell-layer; there are mitoses in these connective tissue cells.

(5) No. 785. Six days, seventeen hours after copulation, extirpate corpora lutea; twelve days later examine uterus and ovaries. The ovaries show that a new ovulation had taken place five to six days previously. There was a cuboidal-cylindrical surface epithelium of the uterus without mitoses. The epithelium of the glands was somewhat higher and also without mitoses. There is a cellular connective tissue under the epithelium with mitoses; underneath the epithelium the nuclei are more densely packed; somewhat further down the tissue is more edematous. The glands and vessels run at right angles to the connective tissue layer.

(6) No. 755. One day, nineteen hours after copulation, left uterus ligatured; extirpate one corpus luteum in left ovary; six days later extirpate one corpus luteum of the right ovary. Examination of ovaries and uterus eighteen days after copulation. Pregnancy was not present. A new ovulation had taken place approximately two days previously, to judge from the condition of the ovaries. The uterus showed high cylindrical epithelium of the surface and of the gland ducts; in both many mitoses were present; some polynuclear leucocytes are in the lumen of some glands. In the connective tissue there are small nuclei present without mitoses. Some cells migrate through the surface epithelium.

We see in these as well as in other similar cases which we need not mention here, a parellelism between the condition of the ovaries and the uterus. An experimentally accelerated ovulation leads to the onset of a new cycle in the uterine mucosa.

IV. The Uterus of Pregnant Guinea-Pigs in which After Excision of the Corpora Lutea a New Ovulation had Taken Place.

As we stated in former papers during pregnancy no new ovulation takes place in the ovary of the guinea-pig.[1] However, after excision of the corpora lutea within the first week after copulation in a considerable number of cases, a new ovulation takes place notwithstanding the presence of pregnancy.[2] We saw that if in a non-pregnant guinea-pig an accelerated ovulation takes place after an early extirpation of the corpora lutea, the uterine mucosa undergoes the typical changes characteristic of the stage of the sexual cycle at which the examination had been made. It is different, if a new ovulation takes place in pregnant guinea-pigs, as the following observations demonstrate:

(1) No. 748. Twenty-one hours after copulation ligature and cut left tube at juncture with uterus, cut out one corpus luteum in the left ovary. Seven days after copulation, cut out one corpus

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[1] "The Cyclic Changes in the Uterus of the Guinea-Pig," *Journal of Morphology*, Vol. 22, p. 37, 1911; *Virchows Arch.*, Vol. 206, 1911.

[2] *Deutsche Med. Wochenschrift*, 1911, No. 1.

luteum of the right ovary. Examination eighteen days after previous copulation shows the presence of embryo and placenta in the right horn of the uterus.

In the left ovary we found young corpora lutea in which a part of the central cavity was still present, while its periphery had been filled by connective tissue; there were small and small to medium-sized good follicles and many follicles in connective tissue atresia. Both uteri show low cuboidal cylindrical epithelium of the surface with vacuoles and narrow glands. The surface epithelium and occasionally the gland ducts near the surface contain some mitoses. The rest of the glands and the connective tissue are without mitoses. The connective tissue is fibrillar and without a cell-layer. Ovulation had taken place approximately three to four days previous to examination (fourteen to fifteen days after copulation). The uterus is typical for pregnancy, but does not show the usual cyclic changes.

(2) No. 752. Ligature left tube near junction with uterus, cut out one corpus luteum from left ovary thirty-three hours after copulation. Seven days after copulation cut out two corpora lutea from the right ovary; examined eighteen days after copulation; pregnancy in right horn of uterus. In the left ovary there were three new corpora lutea, in which the central cavity had been filled by connective tissue; one of the corpora lutea contained a retained ovum in the central cavity; there were small and medium-sized good follicles and various follicles in connective tissue atresia; many follicles were in the last stage of connective tissue atresia. There were some atretic yellow bodies, the remnants of former corpora lutea. The right ovary is similar. A new ovulation had taken place about four to five days previously (thirteen to fourteen days after previous copulation). In the uterus the cyclic changes are absent: we see low cuboidal or low cylindrical surface epithelium with vacuoles which contain chromatin particles and a number of mitoses, narrow glands without mitoses and fibrillar connective tissue rich in nuclei without mitoses. No cell-layer had been formed. The structure of the uterus is the same on the pregnant and non-pregnant side.

(3) No. 780. Six days, six hours after copulation, extirpate

two corpora lutea in left, one corpus luteum in right ovary. Examination ten days later showed the presence of one pregnancy in each horn. The ovaries contained good small and almost medium-sized follicles. There was present one small to medium-sized follicle with a partial, slowly progressing degeneration of the granulosa; there are, furthermore, follicles in various stages of connective tissue atresia. In the right ovary there are two young corpora lutea in which one half to two thirds of the central cavity had been filled by connective tissue. Ovulation had taken place four to five days previously (eleven to twelve days after the previous copulation). The uterus showed low cuboidal epithelium with vacuoles and some mitoses, small glands without mitoses. The connective tissue is fibrillar and contains small nuclei; no mitoses are present in the connective tissue. Cyclic changes in the uterus are absent; the uterus is characteristic of a pregnant guinea-pig.

(4) No. 781. Seven days, five and one half hours, extirpate two corpora lutea in right, one corpus luteum in left ovary. Examination eleven days later; two pregnancies in right horn of uterus; no pregnancy in left horn. In both ovaries there were new corpora lutea in which the central cavity had been filled with connective tissue. The condition of the ovaries indicated a new ovulation about four to five days previously (thirteen to fourteen days after copulation). The uterus shows the same condition as in the former animals (Nos. 748, 752, 780). The epithelium was low, the surface epithelium contained mitoses. The connective tissue did not contain a cell-layer and was without mitoses.

(5) No. 786. Seven days, five hours after copulation, extirpate one corpus luteum in each ovary. Nine days later examination showed one pregnancy in the right horn of the uterus. In both ovaries there were young corpora lutea in which connective tissue began to grow in the periphery of the central cavity; there were small good follicles and follicles in early-medium and late connective tissue atresia. Ovulation had taken place approximately two days previously (fourteen days after copulation). The uterus showed the same structure as in the previous specimens; but the surface epithelium did not contain

mitoses. Vacuoles were however present in small cuboidal cylindricall cells.

(6) No. 789. Seven days, twelve and a half hours after copulation extirpate two corpora lutea in right, one corpus luteum in left ovary. Examination ten days later shows one pregnancy in right, none in left horn. Both ovaries showed young corpora lutea in which the central cavity had been partly or entirely filled by connective tissue. In one of the young corpora lutea an ovum had been retained. There were small and medium-sized good follicles and many follicles in connective tissue atresia. Ovulation had taken place about four to five days previously, about thirteen days after the copulation. The left uterus showed cuboidal epithelium of surface and low epithelium of glands, with only very few mitoses in the surface epithelium; there were occasionally some small vacuoles in the epithelium. The connective tissue was edematous. The blood vessels contained many leucocytes and occasionally a mitosis can be seen in an endothelial cell. Here again the typical uterine changes are absent.

(7) No. 989. Six days, eighteen hours, after copulation excise one corpus luteum in left ovary, excise the entire right ovary without corpora lutea. Examination eleven days later showed one pregnancy in the left horn of the uterusand a young corpus luteum in the left ovary. The center of the corpus luteum was filled with loose connective tissue. There were small and small-medium sized good follicles and many follicles in a medium and late stage of connective tissue atresia. Rupture had taken place about three to four days previously (about fourteen to fifteen days after copulation). The right uterus showed cuboidal-cylindrical epithelium which at some places was somewhat higher. It contained some vacuoles with chromatic material and very few mitoses; the glands were cuboidal-cylindrical and without mitoses. The connective tissue is fibrillar, without mitoses. Cyclic changes are absent.

(8) No. 988. Five days, nineteen and a half hours after copulation excise one corpus luteum in right ovary and extirpate the whole left ovary with two corpora lutea. Examination ten days later. One pregnancy in right horn of uterus. In the right ovary there were young corpora lutea in which the central cavity had

been filled with connective tissue. There were present small and large good follicles. In a few large follicles there was just a beginning of granulosa degeneration. There were many follicles in a late stage of connective tissue degeneration. A new ovulation had taken place about six to seven days previously (eleven to twelve days after the previous copulation). The uterus on both sides showed the absence of a cell layer and of mitoses in the connective tissue which was fibrillar. The surface epithelium was cuboidal or cuboidal-cylindrical, and contained a number of mitoses. The glands showed low epithelium without mitoses.

(9) No. 979. Three days, four hours after copulation excise piece of right uterus. Six days, four hours after copulation excise one corpus luteum in right ovary, extirpate the whole left ovary (with two corpora lutea). Examination twelve days later showed three pregnancies in the left horn. In the right ovary three new corpora lutea had formed not quite three days previously, about fifteen and a half days after the previous copulation. The remaining uterus on the right side showed cuboidal or low cylindrical surface epithelium with a few vacuoles and some mitoses. The glands show cylindrical shape and are at some places higher and contain mitoses. At one place there is more cellular connective tissue with some mitoses in the connective tissue cells present. At this place there had evidently a cut been made at the time of the first operation and it is probable that the abnormality which we find here has been caused either through this cut, or perhaps through an ovum that began to penetrate here into the mucosa but did not continue to develop. However that may be, the changes which we find at one place in the mucosa do not correspond to the stage of the sexual cycle in which the animal was at the time.

(10) No. 987. Five days, nineteen hours after copulation, extirpate right ovary with two corpora lutea, excise one corpus luteum in the left ovary. Examination twelve days later showed two pregnancies in the right horn, apparently none in the left. But microscopic examination disclosed that at one place in the left horn, the ovum had also inserted itself, caused the production of a small decidua, but that the ovum had found difficulty in developing,—perhaps the decidual reaction being not strong

enough. In the left ovary, there were three new corpora lutea present, an ovulation having taken place approximately four to five days previously (thirteen to fourteen days after copulation). The uterus showed low cuboidal or low cylindrical surface epithelium with a few mitoses at some places, glands with cuboidal-cylindrical, not very high cells, without mitoses, fibrillar connective tissue in the mucosa without mitoses, with exception of one place where evidently the ovum had fixed itself and called forth the development of a small decidua with mitotic divisions in the small decidual cells. The typical cyclic changes of the uterus are therefore absent.

(11) No. 967. Three days, three and a half hours after copulation, excise two corpora lutea in the left and three corpora lutea in the right ovary. Examination fifteen days later showed one embryo and placenta in the right horn of the uterus. The left horn appeared thickened. In one ovary we found one, in the other three corpora lutea; their cavity is filled with connective tissue. There are already large good follicles formed, but no granulosa degeneration has as yet taken place. New ovulation occurred about six days previously (twelve and a half days after copulation). The uterus showed at various places the typical structure of the pregnant uterus without formation of a cell-layer. Cyclic changes did not take place in the uterus during pregnancy.

(12) No. 968. Three days, thirteen hours and a half after copulation excise three corpora lutea in the left and one corpus luteum in the right ovary. Examination eighteen and a half days after copulation showed three pregnancies in the right and one small one in the upper part of the left horn. In one ovary two new corpora lutea had been formed about seven or eight days previously (ten to eleven days after the previous copulation). The mucosa of the uterus presented the typical picture of a pregnant uterus.

(13) No. 866. Seven days after copulation excise several corpora lutea both in the left and right ovary. Examination eleven days later. Abortion was in progress in both horns of the uterus. A new ovulation had taken place in one of the ovaries about two to three days previously. The cavity of the

corpora lutea was partly filled by connective tissue. In the uterine mucosa processes of regeneration took place after abortion, polynuclear leucocytes migrated through the mucosa.

(14) No. 673. Four days, two and a half hours after copulation, one corpus luteum was excised in each ovary. At the examination fourteen days later one pregnancy was found in the right horn; in the left horn there was a dilated part. In the left ovary about three to four days previously two follicles had ruptured (fourteen to fifteen days after the previous copulation; new corpora lutea had formed). In the left uterus we found cuboidal to cylindrical epithelium of the surface without mitoses; some epithelium was raised by edematous fluid; the gland ducts were drawn out; the connective tissue cells in the mucosa showed many mitoses. It is possible that an abortion had taken place in the left horn of the uterus and that this complication may explain the cell proliferation in the connective tissue.

(15) No. 1219. Three days, nineteen hours after copulation, one corpus luteum was excised in each ovary. Examination ten days later showed two pregnancies in left, one in right horn of the uterus. In the ovaries we found good follicles of small to medium size, many follicles are in a condition of early or medium connective tissue atresia. In one ovary there is a young corpus luteum with a central cavity; some blood vessels begin to grow into the corpus luteum. Ovulation had taken place about one to two days previously (about twelve to thirteen days after copulation). In the uterus we find cuboidal or cuboidal-cylindrical surface epithelium in which there are vacuoles caused by immigrated cells. There are some rare mitoses in the surface epithelium. The epithelium of the glands is low cuboidal or cuboidal to cylindrical and is without mitoses. The connective tissue is fibrillar and contains small nuclei; no mitoses are present. The early pregnancy prevents the cyclic changes in the uterus.

We see, therefore, that during pregnancy the typical cyclic changes as we described them above do not take place. Instead we find on the whole the characteristic structure of the pregnant uterus. There are two or three specimens in which some doubt about the interpretation of the structural condition of the uterus might arise. It is probable that in those cases secondary changes, due to embedding of an ovum which was not followed by normal embryonic development, had taken place in the mucosa. Only in one case there might be some doubt as to whether the typical cyclic changes had not taken place in the uterus notwithstanding the presence of pregnancy.

As to the way in which pregnancy influences the uterine mucosa, it is probable that the activity of the living decidual cells prevents the proliferation of the uterine mucosa. It cannot be a mechanical effect of pull on the uterine wall thourgh the ovisac because the uterus of a nonpregnant horn behaves in a similar manner as the pregnant horn. If ovulation takes place after an experimentally produced placentoma has become necrotic the typical cyclic changes of the uterus follow ovulation, as in the following experiments:

No. 1152. Six days, twenty and one half hours after copulation, cuts were made into the uterine wall; examination nineteen days later (twenty-five days, twenty and a half hours after copulation). In the ovaries we found just ruptured follicles. There were present corpora lutea which were beginning to become vacuolar and to shrink (the corpora lutea of the former generation). Small good follicles as well as follicles with granulosa degeneration into which connective tissue began to grow were seen. These conditions indicated that ovulation had taken place within the previous twenty-four hours. The left uterus above the nectoric placentoma showed high cylindrical epithelium with a hyaline basal area. At some places the epithelium is lower as a result of pressure. The glands have high or medium cylindrical epithelium. There are some dilated gland ducts filled with polynuclear leucocytes. No mitoses are present in the surface epithelium and only a few in the gland ducts. At some places polynuclear leucocytes migrate into the surface epithelium and disintegrate into small chromatin particles. The structure of the uterus corresponds therefore to the condition found approximately within the first thirty-six hours after ovulation. The condition of the ovaries and of the uterus correspond to each other.

Also in another guinea-pig (No. 1140) the presence of necrotic placentomata in both horns of the uterus did not prevent the development of the typical cyclic changes in the uterus. In this case cuts had been made into both horns of the uterus six days, six and a half hours after copulation. Nineteen days later, twenty-five days, eight and a half hours after copulation, we find on examination the presence of necrotic placentomata. Some small areas of the placentoma were still alive. Condition of the ovaries showed that ovulation had taken place about five days previously. Correspondingly we find in the uterine mucosa a cell layer with mitoses in connective tissue cells and some mitoses in gland fundi. The surface epithelium is cuboidal to cylindrical and contains some mitoses, perhaps as a result of the regenerative changes which are taking place at that time.

Pregnancy seems also to prevent the changes in the uterus which accompany the period of heat. This we found in guinea-pig No. 1300 in which three days, eighteen hours after copulation the thyroids had been extirpated. Examination seventeen days, nineteen and a half hours after copulation showed that this did not interfere with the development of the typical cyclic changes in the ovaries. They present the picture characteristic of this period. There are present several fully matured follicles ready to rupture, but the corpora lutea of pregnancy which do not show any sign of vacuolization and have vessels with well developed walls, prevent this rupture. We should expect under those conditions (period of time, presence of mature follicles) in a non-pregnant uterus high cylindrical epithelium of the surface and of the gland ducts, lower gland fundi, the nuclei of the connective tissue somewhat large and vesicular. Instead, we find the typical picture of the uterus during pregnancy; low cuboidal epithelium of the surface with a few isolated mitoses, low glands without mitoses, fibrillar connective tissue with small nuclei. At some places transudate raises the surface epithelium from the underlying connective tissue. Is it not probable that extirpation of the thyroids prevents the uterine changes accompanying heat because we observed the occurrence of these changes in another guinea-pig in which the thyroid had been removed previously. We may, therefore, conclude that the presence of pregnancy prevents those changes in the uterus which accompany the period of heat. And it appears probable that pregnancy prevents also the psychical changes characteristic of the state of heat.

V. The Influence of Extirpation of the Corpora Lutea on the Duration of the Cyclic Period.

I have previously shown that the duration of the sexual period in the mammalian female depends upon the duration of life of the corpus luteum.[1] The corpus luteum prevents a new ovulation and the onset of a new sexual cycle.

In our former experiments some operative interference had usually taken place in the case of the control guinea-pigs as well as in those in which the corpora lutea had been excised. It was therefore necessary to determine the length of the sexual cycle in the normal guinea-pig. This can be done by observing the time of the heat of a guinea-pig and by examining uterus and ovaries at known intervals. Under those conditions we found the length of the sexual period to be usually sixteen to eighteen or nineteen days; sometimes the new ovulation may take place as early as fifteen days after copulation. In two exceptional cases we observed the new ovulation as early as thirteen and a half to fourteen and a half days. If we allow copulation to take place and ligature the tubes at their juncture with the uterus, and in addition, the upper part of the uterus, within the first two and a half days after copulation, the new ovulation also may take place between the sixteenth and eighteenth day, but in other cases it occurs later. If we make incisions into the uterus throughout its whole length five to seven days after copulation, ovulation takes place in the majority of cases between the twentieth and thirtieth day after copulation. At the times ovulation occurs the corpora lutea of the previous sexual period have begun to degenerate and the experimentally produced placentomata have to a great extent become necrotic. The beginning degeneration of the corpora lutea is a prerequisite for the new ovulation. If, after copulation, pregnancy is permitted to take its normal course, no ovulation occurs until birth has taken place. If abortion has taken place, a new ovulation also can occur.

If we excise the corpora lutea within the first week after copulation, a new ovulation occurs in the majority of cases between the tenth and fifteenth day after copulation. We observed it as early as eight and a half and nine days after copulation. Pregnancy does not prevent the early ovulation after a complete extirpation of the corpora lutea. It is only the persistence of the corpora lutea of pregnancy which prevents the ovulation. Pregnancy however prevents, as we saw, the cyclic changes in the uterus which accompany and follow ovulation. On a former occasion I suggested that embryonic structures determine the persistence of the corpora lutea during pregnancy.[1] We are at the present time testing this hypothesis experimentally.[2] If we extirpate the corpora lutea incompletely, no new ovulation takes place.

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[1] *Zentralblatt f. Physiologie*, Bd. XXIII., No. 3, *Deutsche Med. Wochenschrift*, 1911, No. 1.

These experiments, as well as those mentioned above which have already been partly published, and which as far as they concern new problems shall be published more in detail in the near future, clear up to a great extent the mechanism of the sexual cycle.

VI. The Signs of Heat in the Guinea-Pig.[3]

(Report by Miss A. E. C. Lathrop.)[4]

"When a number of females are kept together in a large pen, some will be seen sitting quietly, some eating a little, or walking about, making a small, sociable clucking noise.

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[1] *Zentralblatt f. Physiologie*, Bd. XXIV., No. 6.

[2] We produced recently experimentally an early stage of extrauterine pregnancy in a guinea-pig. The presence of some living embryonic structures and of fetal placenta did not prevent the degeneration of corpora lutea and the subsequent rupture of follicles. On the other hand the corpora lutea persisted as long as experimentally produced placentomata (structures of maternal origin) remained alive in cases in which we made incisions into the uterus six or seven days after copulation. In some of these cases fetal structures developed in these placentomata; in other cases no fetal structures could be found. It is therefore possible that the maternal placenta determines directly the life of the corpora lutea and that the embryonic structures act only indirectly by prolonging the life of the placentomata. We are testing experimentally this theory at the present time.

[3] The condition of heat in the majority of the guinea-pigs which we used was observed by the breeders, Mr. J. M. Simpson in Colwyn, Pa., and Miss A. E. C. Lathrop in Granby, Mass. Dr. O. Ishii also observed the condition of heat in a number of cases. I had an opportunity to control the correctness of these observations by the microscopic examination of ovaries and uterus. In almost all cases I found the observations of the breeders confirmed by the microscopical examination.

[4] I asked Miss A. E. C. Lathrop to observe the signs of heat in the guinea-pig and she sent a statement of her observations which, with her permission, I wish to incorporate in my paper after some slight changes have been made. She writes as above.

"If one moves occasionally, rather majestically, among the rest, making a low, purring noise, similar to the noise of the male, she is probably in heat; if not, she soon will be. In some cases, a female guinea-pig may reach a state of excitement in which she assumes the manners of a male and attempts copulation with another guinea-pig which happens to be in heat. She, as well as the second guinea-pig that allows her to attempt the rôle of a male, will almost always be found ready for copulation.

"In many other cases, however, no such clear indications are present, but on close observation it will be seen that some female which is sitting quietly will be found to elevate slightly her hips whenever another guinea-pig happens to touch her. Again other females will show no sign of their condition, unless an active male is put among them. Those in heat take interest in the male, when he comes near them, while those not in heat or near the period of heat will kick him and hop away, making a little complaining noise when he pays attention to them. The male has no means of selecting the females which are in heat. When he has accidentally found one, he cannot find her again a moment later, but must try every female which he happens to come near to until he by chance meets again the animal in heat. Neither do the females that are in heat go to the male, but they wait, wherever they happen to be, until he comes to them. Some females will not give any indications whatever of their condition, but only when the male attempts copulation will they be found to be in heat.

"There are occasionally individuals which always appear to be in heat; at frequent intervals when not busy eating they purr and walk about like a male, but never seem to be actually ready for copulation. They resent the advances of the male at all times and yet are not sterile; when left for some months in a lot of males and females they may become pregnant.

"When one or very few females are kept in a small cage and have become relatively tame, those in heat when tickled along flanks and hips will react to the touch of the fingers, assuming a position suitable for copulation.[1] This sign is, however, of no use when the animals are timid or when a number of the females are kept in the pen; under those conditions they pile up in a corner when one tries to touch them.

"The condition of the vulva may be of value in determining the state of the female. If a drop of mucus can be seen in the vulva, the animal is not in heat, even if acting somewhat excitedly, but it will be found in heat within a few hours. At the times of heat we find usually a little moisture or almost dried mucus at the vulva and a slight enlargment or swelling of the entrance to the vagina which may be so slight that it becomes noticeable only if the animal is compared with others.

"In some, perhaps in most cases, a day or two before heat the animal while not showing any of the above-mentioned signs, may be unusually lively and playful, and when taken in hand and brushed very lightly over pelvic region, we notice a twitching about the pelvis and especially the vulva."

VII. Effect of Extirpation of the Ovaries on the Cyclic Changes in the Uterus.

We found that extirpation of the corpora lutea shortens the sexual cycle. It does not to any noticeable extent influence the various stages in the development of the ovarian follicles, but it causes an early rupture of mature follicles. Extirpation of the corpora lutea changes however the cyclic changes in the uterus. It prevents the predecidual proliferation of the connective tissue cells of the mucosa and modifies also to some extent the cyclic changes in the uterine epithelium. It does not however prevent those changes in the uterus which accompany heat and precede ovulation. The existence of a corpus luteum is required only as far as a certain part of the cyclic changes in the uterus is concerned; certain other changes (those preceding ovulation) are in all probability in a similar manner as ovulation itself inhibited by the existence of a functionating corpus luteum. However, if we extirpate both ovaries instead of the corpora lutea, all cyclic changes in the uterus come to a standstill.

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[1] A similar observation had been previously communicated to me by Mr. J. M. Simpson.

Several months after castration, the wall of the uterus is thin; the surface epithelium is cuboidal or low cylindrical. There are mitoses present in the surface epithelium; their number varies. The glands are small and without mitoses; the connective tissue of the mucosa is fibrillar, contains small nuclei and is somewhat richer in nuclei directly beneath the epithelium than in the deeper parts of the mucosa. Two to three weeks after castration we find also low cuboidal or low cylindrical epithelium covering the mucosa of the uterus; no mitoses are present in the gland cells which are all small. The connective tissue is fibrillar and the connective tissue nuclei are small and without mitoses.

We see that after total extirpation of the ovaries the mucosa of the uterus becomes similar to the mucosa of the pregnant uterus in a non-pregnant horn. Gradually all the layers of the uterine wall seem to become thinner.

If we extirpate the ovaries within the first twenty-four hours after copulation and examine the uterine mucosa six or seven days after copulation we find the surface epithelium usually low cuboidal or cylindrical; it may, however, be somewhat higher; there are mitoses present in the surface epithelium; the glands have low epithelium and are usually without mitoses. In the connective tissue no cell-layer developed and no mitoses are visible. We find, therefore, here a similar result as after extirpation of the corpora lutea, namely, inhibition of the connective tissue proliferation and at the same time continued mitotic proliferation in the surface epithelium, which is lacking at this period in the normal uterine cycle. The extirpation of the ovaries acts in this respect principally through the lack of the corpora lutea which castration entails. In one case, however, in which fresh corpora lutea of the guinea-pigs were injected into a guinea-pig which had been castrated sixteen and a half hours after copulation, five days after castration a few mitoses were found in the connective tissue of the mucosa. We must leave it at present undecided whether the injection of the corpus luteum substance produced this result.

If we extirpate within the first twenty-four hours following copulation the ovaries and make incisions into the uterus six to seven days after copulation we find on examination ten days after the last operation the absence of placentomata in the uterus. Mitoses are, however, present in the surface epithelium and sometimes also in the gland cells. Twice we found a few mitoses in the connective tissue of the mucosa which may either be myxoid in character or show the usual fibrillar character. Just as early extirpation of the corpora lutea prevents the experimental placentomata, so does extirpation of the ovaries have the same effect and we may, therefore, assume that also in this case extirpation of the ovaries is effective through the accompanying removal of the corpora lutea.

Through lack of the corpora lutea the sensitizing or preparing substance is removed which in combination with the mechanical factors causes the predecidual cell proliferation and the development of the placentomata. Under these conditions the cuts do not cause proliferation in the connective tissue of the mucosa.

If we extirpate the ovaries six to seven days after copulation and at the same time make incisions into the uterus, we may find on examination ten to thirteen days later either none (two cases), or very small placentomata (in one case in which the examination had been made thirteen days after excising the uterus). In the last mentioned case, about one half of the placentoma had become necrotic, the remaining part showed a hyaline character with nuclei embedded in a homogeneous glassy matrix. At one place there were still visible in the placentoma parts of the fetal placenta in the form of syncytiomatous vessels passing into the maternal tissue and also in the surrounding fat tissue. The extirpation of the ovaries caused in this case a very incomplete and retarded development of the ovum; a similar retardation in the development of the ovum we found also in a case of extrauterine pregnancy and a similar effect may be observed after extirpation of the corpora lutea. Under these conditions the soil is evidently not favorable for the development of the ovum.

In three cases in which the ovaries were extirpated eight days or almost eight days after copulation and at the same time incisions were made into the uterus, very small placentomata developed in two cases and only a trace of a placentoma in one case. On examination eleven days after the operation, a partial necrosis was found in the placentoma; no mitoses, or only exceptional mitoses were found. Under certain conditions we observed however in the placentoma an amitotic proliferation of nuclei which may lead to the formation of giant cells. The living parts of the placentomata were hyaline and partly vacuolar.

Extirpation of the ovaries six to eight days after copulation does not prevent, therefore, entirely the production of placentomata through incisions made into the uterus simultaneously with the castration, but it reduces very much the cell proliferation and may cause a partial early necrosis.

We found also that extirpation of the corpora lutea six to eight days after copulation led to the production of placentomata which were smaller than usual. It is however possible that extirpation of the ovaries has a still more strongly inhibiting action on the growth of the placentomata than removal of the corpora lutea.

In several other series of experiments the ovaries were extirpated at periods following the incisions into the uterus. If the incisions into the uterus were made about six days after copulation, the ovaries extirpated two days later and the examination made eight or ten days after the last operation, placentomata developed in all cases, but their size varied. In one case they were of medium size, but usually they were smaller than normal. The amount of necrosis and hemorrhage also varied; these changes could be very insignificant or they could be very considerable.

In another series incisions were made into the uterus about six days after copulation; seven days later the ovaries were extirpated and seven days after castration the examination was made. In these experiments the placentomata were therefore fourteen days old at the time of examination, and the ovaries were extirpated at a time when the placentomata had already formed. The placentomata were found almost entirely necrotic; some small living areas were present usually directly under the outer surface of the placentoma or near the mucosa,—both places where the nourishment was still better. No mitoses were seen in the living part of the placentoma.

In the surface epithelium of the mucosa and in the glands of the uterus mitoses were found. In one case mitoses were also found in the myxoid connective tissue of the mucosa and in this case mitoses in the glands were usually present at places in the neighborhood where also the connective tissue showed mitoses. In other cases, however, mitoses were only found in the surface epithelium and glands, but not in the connective tissue. These changes in the mucosa are in all probability to be interpreted as regenerative in character, following the necrosis of the placentoma.

We may, therefore, conclude that extirpation of the ovaries does not prevent regenerative proliferation in the mucosa of the uterus; furthermore that extirpation of the ovaries shortens in all probability the life of the placentomata.

In a further series incisions were made into the uterus six to seven days after copulation; ten days later the ovaries were extirpated, and after a further lapse of five days (fifteen days after incising the uterus) the examination was made. In these cases the ovaries were therefore extirpated at a still later period and the examination was made at a somewhat earlier date after castration. The placentomata were found to a great extent necrotic; some smaller parts which were better nourished, near the mucosa and around large blood vessels were usually still alive; no mitoses were found in the living cells. Here again we find proliferative processes in the surface epithelium and in three out of five cases also in the gland ducts of the uterine mucosa near the necrotic placentomata. The gland cells in those ducts in which mitoses are found are higher. Even in the connective tissue of the mucosa there may be a few mitoses in the connective tissue cells or in the blood vessels. This mitotic proliferation must in all probability again be interpreted as regenerative in character. Castration does therefore not prevent these regenerative changes in the mucosa of the uterus.

In control experiments in which incisions were made into the uterus six to eight days after copulation but in which the ovaries were left intact, the placentomata were still alive thirteen days, and in some cases partly alive eighteen and nineteen days after the incisions had been made. In other cases they were entirely necrotic eighteen to twenty-four days after incising the uterus.

If, however, only one horn of the uterus had been incised and pregnancy proceeded in the other horn the placentomata were invariably found alive even as late as twenty-five days after incising the uterus.

We see that the function of the ovaries depends partly upon the presence of functionating corpora lutea; the latter cause the predecidual proliferation of the connective tissue of the uterine mucosa, are a necessary factor for the production of the placentomata and determine directly or indirectly at certain periods of the sexual cycle, the size, shape and mitotic proliferation of the epithelial cells of the uterine mucosa. Other parts of the changes in the uterine mucosa, namely, those accompanying or directly preceding heat are determined by other parts of the ovaries and not by the corpora lutea. They take place after extirpation of the corpora lutea; they lead indeed to the formation of new corpora lutea; they are however prevented through the extirpation of the ovaries. Which part of the ovaries is responsible for these changes characteristic of heat cannot be easily determined experimentally but can only be surmised. On a former occasion we stated the reasons why we thought it very improbable that the so-called interstitial gland of the ovary is responsible for the cyclic changes accompanying heat.[1] In the first place in the ovary of the guinea-pig there does not exist a structure deserving of the name "interstitial gland." We merely find small shrinking connective tissue cells of theca interna filling the place of lost parts of the follicles which are in process of atresia; secondly the quantity of follicles in late stages of atresia is perhaps greater five to six days after ovulation than at the period of heat, There is usually towards the end of a cyclic period a considerable number of such follicles present in the ovary, but in other cases, especially in younger animals, the number present may be relatively small.

The only structure which is, so far as we know, invariably connected with the presence of heat and which originates at the time preceding heat is the mature follicle. The granulosa cells of a mature follicle differ in some of its morphological and physiological characteristics from those of ordinary growing follicles.[2]

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[1] *Centralblatt f. Physiologie*, Bd. XXV., No. 9.

[2] *Journal of Morphology*, Vol. 22, March, 1911; *Virchows Arch.*, Bd. 206, 1911.

It is therefore probable that they are in some way responsible for the changes in the uterine mucosa accompanying heat. Even if extirpation of these follicles should not prevent the occurrence of heat, this would not be conclusive evidence against this assumption inasmuch as the mature follicles had already had an opportunity at the time of extirpation to exert their influence. In a similar manner we have shown previously that extirpation of the corpora lutea five to six days after ovulation does not prevent the production of placentomata, while extirpation done one day after ovulation has a preventive effect. There still remains of course the ordinary follicles as the possible source of the energy leading to these changes in the uterine mucosa; inasmuch as they are present equally at other times of the sexual cycle, while the mature follicles are present only at certain periods and especially at the time of heat or immediately preceding heat, it is more probable that the mature follicles are responsible for these changes. Whatever the part of the ovary may be that brings about these changes, there must be added to this positive factor two negative ones, namely the absence of a fully functionating corpus luteum and of pregnancy, these conditions being necessary for the development of the normal and complete sexual cycle.

As we saw, the ovaries have still another function, namely that of maintaining the full size of the uterus. After castration various constituent parts of the uterine wall diminish in size.

VIII. Summary.

1. The cyclic changes in the epithelial as well as connective tissue structures of the uterus during heat preceding copulation and the changes following copulation are described; the influence of polynuclear leucocytes in these changes is shown. The latter are present during the first two days following copulation. The decidual cells originate from the connective tissue cells of the mucosa.

Various periods of the sexual cycle are characterized through the shape and mitotic proliferation of various epithelial and of the connective tissue cells. From the sixth to the twelfth day after ovulation mitoses are usually absent in the epithelial structures; afterwards they begin to reappear, from the eighth day on to the end of the sexual cycle mitoses are absent in the connective tissue.

2. As long as the corpora lutea functionate, the presence of mature follicles in the ovaries does not produce those changes in the uterine mucosa which are characteristic of the period of heat.

3. During pregnancy the structure of the uterus is that of a resting organ, in which epithelial as well as connective tissue cells are small and the latter are without mitotic proliferation.

4. In animals in which heat has been observed without copulation taking place, the cyclic changes in the uterus are approximately the same as in animals in which copulation did take place, in which however pregnancy had been prevented through an early ligature of the fallopian tubes. The main difference in these two series is caused through the very marked migration of polynuclear leucocytes through the mucosa in cases in which copulation took place.

5. Ovulation is usually accompanied or directly followed by certain changes in the uterine mucosa. These changes permit us in many cases to decide whether or not in an animal in the period of heat ovulation has or has not yet taken place.

6. Extirpation of almost the whole uterus or thyroids does not prevent the occurrence of heat in the operated animals.

7. In animals which were prevented from copulating or in which the fallopian tubes were ligated soon after copulation, the period of heat occurs approximately every fifteen to nineteen days.

8. The proportion of ovarian follicles which are in the late stages of connective tissue atresia to well preserved follicles and follicles in the stage of granulosa degeneration shows two maxima, one about six to seven days after ovulation and another at the time preceding the next ovulation. Under certain conditions, however, the number of quite atretic follicles may be small at the time of heat, namely in young guinea-pigs which are in heat for the first time, and it may be relatively large at other periods of the sexual cycle in old guinea-pigs.

9. As we showed previously an early extirpation of the corpora

lutea leads in the large majority of cases to an acceleration of the next ovulation. Certain conditions, however, may prevent the early ovulation. In the latter cases the structure of the uterus corresponds to the late stage of the sexual cycle. If however a new premature ovulation is brought about through the extirpation of the corpora lutea, a new cycle starts in the uterus at the time of the accelerated ovulation. Mature follicles, or possibly other follicles, are in the absence of corpora lutea able to call forth the structural changes in the uterus accompanying heat and the rupture of the follicles and development of corpora lutea is followed by the typical uterine cyclic changes.

10. We stated previously that also during pregnancy extirpation of the corpora lutea is followed by an early ovulation. While thus pregnancy in itself does not prevent an ovulation to take place (only the corpora lutea persisting during pregnancy preventing the ovulation) pregnancy does prevent the cyclic changes of the uterus preceding, accompanying or following ovulation; during pregnancy the uterus retains its resting condition notwithstanding ovulation. This influence of pregnancy is not a mechanical one, inasmuch as it is found in the non-pregnant horn as well as in the pregnant horn in cases in which a pregnancy developed only in one horn. The presence of a necrotic placentoma or the occurrence of an abortion does not prevent the cyclic changes in the uterus that set in with ovulation.

11. If five to seven days after copulation both horns of the uterus are split lengthwise and the development of a normal pregnancy is prevented, ovulation is delayed under those conditions; it occurs between the twentieth and thirtieth day after the previous copulation, even in cases in which a developing embryo could not be seen in the placentomata growing at the site of the incisions. On the other hand in a case of an experimentally produced extrauterine pregnancy degeneration of the corpus luteum and a new ovulation were not delayed notwithstanding the presence of living embryonic (including placental structures of embryonic origin) in the animal. These observations suggest that it is primarily the life of the maternal part of the placenta which prevents the new ovulation during pregnancy and that the embryonic structures are only indirectly concerned

in this effect. We are testing this suggestion still further at the present time.

12. A description of the signs of heat is given in accordance with the observations of the breeder, Miss A. E. C. Lathrop.

13. After total extirpation of the ovaries, the uterine mucosa becomes similar to the mucosa of the pregnant uterus in a non-pregnant horn; gradually an atrophy of the different layers of the uterine wall takes place. If we extirpate the ovaries soon after ovulation the effect is the same as if the corpora lutea are extirpated at that period, as far as the predecidual proliferation in the connective tissue and the accompanying changes in the epithelium which take place between the fourth and seventh day are concerned. As far as this effect is concerned the corpus luteum is the active part of the ovaries. While, however, extirpation of the corpora lutea does not prevent those uterine changes which accompany heat and does not prevent a new sexual cycle, extirpation of the ovaries prevents all cyclic changes in the uterus.

14. Early extirpation of the corpora lutea within the first forty hours after copulation prevents in a similar manner the development of experimental placentomata as an early extirpation of the ovaries. If the ovaries are removed six to eight days after copulation and at the same time incisions are made into the uterus, either none, or only very small placentomata develop and the developing placentomata die at an earlier date than they would have done otherwise. An extirpation of the corpora lutea six to seven days after copulation also causes the developing placentomata to be smaller. If under these conditions an ovum develops in the incised uterus within the decidual tissue, the development of the ovum is usually much retarded. A similar retardation of the development of the ovum we observed in a case of experimentally produced extrauterine pregnancy in the guinea-pig. If the ovaries are extirpated two days after the making of the incisions the developing placentomata are usually smaller. Castration done at a time when the placentomata had already reached a considerable size causes an acceleration in the necrosis of the placentomata. Castration does however not prevent regenerative changes in the uterine mucosa.

15. The existence of pregnancy in one horn of the uterus prolongs markedly the life of placentomata produced in the other horn.

16. For certain phases of the cyclic changes of the uterus the presence, for other phases the absence, of a functionating corpus luteum is necessary; but certain other ovarian structures (probably mature follicles and not the so-called interstitial gland) are required for the latter phases. In addition the absence of pregnancy (and probably of living placentomata) is a condition for the typical course of the cyclic changes in the uterine mucosa.

A PRELIMINARY ACCOUNT OF THE CHROMOSOMES IN THE EMBRYOS OF ANASA TRISTIS AND DIABROTICA VITTATA.

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It was suggested to the writer, in 1912, by Prof. E. G. Conklin that a comparative study be made of the chromosomes in the somatic cells of certain insects, as contrasted with the sex cells. The first material was collected during the following summer, and included embryos of *Anasa tristis* and *Diabrotica vittata*. Material of a number of other forms, not restricted to the Insecta has since been collected and examined to some extent. The results obtained from the latter, together with a more extended account of the forms briefly described here, will be discussed at greater length in another paper.

Embryos of *Anasa tristis* were obtained covering a period of development from the incomplete blastoderm stage up to about the time of hatching. Some difficulty was experienced in obtaining the eggs of *Diabrotica vittata*, but fortunately the stages obtained extend from the second maturation division up to the segmentation of the embryo.

Anasa tristis.—In 1906, Wilson in his "Studies on Chromosomes," Part III., gave the results of his work on the spermatogenesis of *Anasa tristis*. There are twenty-one and twenty-two chromosomes in the spermatogonia and oögonia, respectively. Of the twenty-one chromosomes in the spermatogonia, twenty can be paired. There is, here, a marked differentiation in the size of the chromosomes. In each plate there are three large or macrochromosomes, and two small or microchromosomes. The unpaired or *x*-chromosome (idiochromosome; accessory chromosome) is one of these three largest chromosomes, although it cannot be exactly determined which one it is. The two microchromosomes have been termed the *m*-chromosomes. In the second maturation division the *x*-chromosome moves undivided to one pole so that one half the number of the spermatids receive eleven, the other half, ten chromosomes. Each spermatid also receives one *m*-chromosome.

In the oögonial group there are twenty-two chromosomes, which can be arranged in eleven pairs. Of these, four are macro- and two are microchromosomes. Wilson was able to count the chromosomes in the ovarian follicle-cells. These were found to be identical in number with the chromosomes in the oögonia. He says, however, that "not infrequently the number of chromosomes is much greater, and the same is true of the nuclei of the investing cells of the ovary, of the oviduct and the fat-body. In the male similar multiple groups are not uncommon in the interstitial and investing cells of the testis. Only in a single case have I succeeded in gaining a clear and complete view of such a group; but this one case suffices to give, with a great degree of probability, the explanation of the increased number of chromosomes. In this case every chromosome is exactly twice the oögonial number, namely 44." This figure, which he gives, is from a cell toward the periphery of a larval ovary, and shows eight macro- and four microchromosomes, twice the number of these particular chromosomes found in the oögonia. He suggests that the chromosomes have divided once without a corresponding division of the cell-body, and he thinks it probable that an increase in number of chromosomes in these particular cells is always due to a process of this kind.

Wilson also states that although he was unable to obtain perfect preparations of mitoses in other tissues, he is able to assert that in the ectodermal cells of the larva the number of chromosomes is "approximately the same as in the oögonia," and that nowhere else than in the described cases did he obtain a doubling of the number. He concludes that this multiplicity is due, perhaps, either to the fact that the cells in question are degenerating, or that they are highly specialized.

In 1910, Morrill published his observations on the chromosomes in the oögenesis, fertilization, and cleavage of certain Coreid Hemiptera, among them *Anasa*. He found that the oögonial groups in *Anasa* contain twenty-two chromosomes, including four macro- and two microchromosomes. The reduced number in the matured egg he found to be eleven. The chromosomes in the reduced groups, either in the polar body or the egg, showed the same relative size differences as the corresponding pairs in the oögonia, and the chromosomes in the matured ova correspond, in general, to those of a sperm bearing the *x*-chromosome. The chromosome groups, which were counted in the embryonic mitoses of *Anasa* were from incomplete blastoderm stages. The embryos were found to be of two kinds: one containing twenty-one chromosomes, the other twenty-two. One case was reported of twenty-three chromosomes, but this Morrill suggests may be due to an accident in technique. The two different chromosome groups, those having twenty-one and twenty-two, correspond, respectively, to the groups found in the spermatogonia and the oögonia. There are the same size differences, and the same number of macro- and microchromosomes. He states that the chromosomes in general are more elongated in the embryonic mitoses than in those of the germ cells, which makes the task of pairing them extremely difficult, if it is at all possible, except in the case of the macrochromosomes and the two *m*-chromosomes.

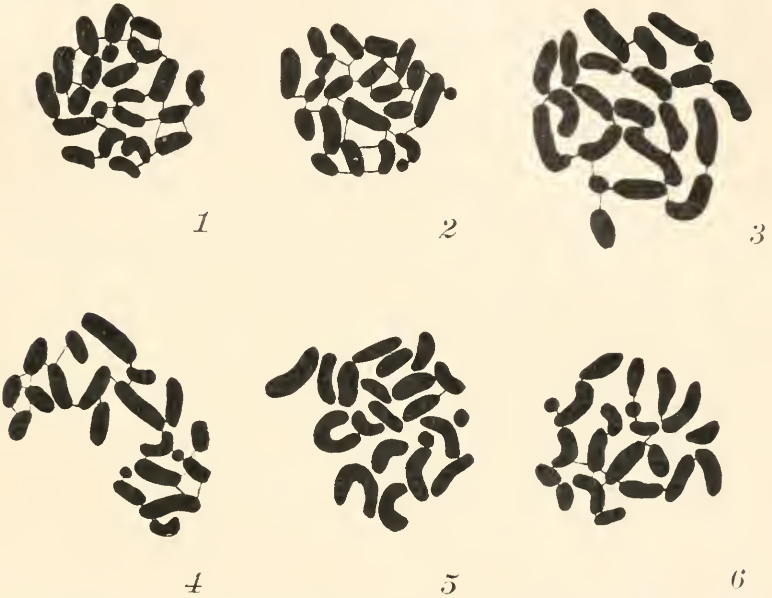
The results, which the present report embodies, were obtained, in the case of *Anasa*, from a study of the somatic mitoses in several stages of development. Although mitoses are abundant in all the tissues of the embryos, few are sufficiently favorable for even tentative counts, still fewer for actual counts. In no case has it been possible to obtain more than seven counts from one individual, while three counts are more nearly the average. The chromosomes, especially in the metaphase groups, are crowded together, and the possibility of counting them at all depends chiefly on the extent of destaining.

At first some rather startling results were obtained. Some counts revealed a number as low as fourteen chromosomes in one plate, while others ran up to twenty-four in number, and there seemed to be no number characteristic of any one tissue, though the average varied around eighteen and twenty. When, however a large number of series was examined, and the exact point of optimum destaining was acquired, the results tabulated showed that the seemingly aberrant counts were wrong, and that they might be due to any of the following reasons; (1) counting early prophases, where the chromosomes lie at different levels, and are crowded and overlapped; (2) counting plates, one or several chromosomes of which lay in an adjoining section (though this does not mean that such figures are necessarily valueless); (3) some chromosomes were torn out of the section; (4) one or more chromosomes had been cut by the microtome knife, and the resulting ends or segments appeared in the next section; (5) the destaining of the slide had not progressed far enough to differentiate, one from another, some of the chromosomes lying quite close together.

Approximately one hundred groups of chromosomes from different tissues, and from a large number of embryos, have been drawn and counted. The stages, extending from the incomplete blastoderm stage up to about the time of hatching, which have been examined, are at a period of more or less high differentiation, when any chromosomal differentiation would most likely be apparent, if this is a regular concomitant of cell differentiation. The results so far show most conclusively that there is no such differentiation in the number of the chromosomes in *Anasa*, and that the embryos are of two classes as to their chromosome number, one having twenty-one, and the other twenty-two chromosomes in all the somatic cells. Moreover, the same general size differences in the chromosomes may be observed in these comparatively late stages of development as are apparent in the spermatogonia and the oögonia.

The accompanying figures serve to illustrate these points. The three upper figures (Figs. 1, 2 and 3) are from embryos having twenty-two chromosomes, and are at a stage of development where the limbs are quite elongated and the embryo as a whole has not shortened up. Figs. 1 and 2 are from the hypodermal layer of the antenna, and are from the same embryo. In both figures it will be noted that there are four chromosomes larger than the others, though this difference in size is perhaps clearer in Fig. 2. In each of the figures, again, the two *m*-chromosomes appear. Fig. 3 is from a cell in the cerebral ganglion of another embryo. These cells are much larger than any others in the embryo, consequently the chromosomes are much larger. The *m*-chromosomes are easily seen, and one pair of chromosomes is distinctly larger than the others. The second pair of macrochromosomes is somewhat more difficult to make out.

The three lower figures (Figs. 4, 5 and 6) are from embryos with twenty-one chromosomes. Figs. 4 and 6 are from mesoderm cells of two different embryos, at a stage when the limb buds are first appearing. Fig. 5 is from a cell in the cerebral ganglion of another individual, at a much later stage of development. In each of these figures the *m*-chromosomes are easily distinguishable, and it can be seen that there are two paired and one unpaired macrochromosome, particularly well shown in Fig. 4. Although no attempt has been made to pair the chromosomes, in several of the figures shown it would be quite possible to do so.



Anasa tristis. Initial magnification of 4,800 diameters, reduced one third. Figs. 1 and 2, hypodermal layer of the antenna, 22 chromosomes. Fig. 3, cerebral ganglion cell, 22 chromosomes. Fig. 4, mesoderm cell, 21 chromosomes. Fig. 5, cerebral ganglion cell, 21 chromosomes. Fig. 6, mesoderm cell, 21 chromosomes.

In all the cases studied the twenty-one chromosome groups generally show quite clearly three chromosomes larger than the rest, while the twenty-two chromosome groups show four such chromosomes. In most of the figures the *m*-chromosomes stand out clearly. In a few cases it is difficult to make them out, owing to the fact that they lie very close to a larger chromosome.

Of course a difference in the general size of the chromosomes as a whole is to be found, and it is probable that no two groups would appear exactly alike. The size of the chromosomes in the metaphase groups is directly proportional to the size of the cytoplasmic body. In the metaphase groups there is a longitudinal splitting of each chromosome. In no case has a transverse division been observed here, nor has there been observed a doubling in the number of chromosomes in this phase, such as Wilson describes in the follicle cells.

Diabrotica vittata.—The spermatogenesis of *Diabrotica vittata* was worked out and published in 1907 by Miss Stevens. In the spermatogonial divisions she found twenty-one chromosomes of various sizes and shapes. The *x*-chromosome which she figures is one of the medium-sized chromosomes. The other twenty she was able to pair. In the second spermatocytic division the *x*-chromosome appears as a more or less rounded body, which passes undivided to one of the poles of the spindle. There are no such clear cut size differences as are found in *Anasa*.

The results which I have obtained from a study of the eggs and early embryos of *Diabrotica*, though somewhat meager it is true, clearly support the same conclusion as was reached in the case of *Anasa*, namely that the embryos are of two classes, one having twenty-one, and the other twenty-two chromosomes in all the mitoses.

Some clear cut figures were obtained in the maturation of the egg. The anaphase of the first maturation division revealed eleven dyads. These in turn divided, leaving eleven monads in the fully matured egg. This brings the maturation of the egg of *Diabrotica* into line with that described for other insects.

In conclusion it may be said that in neither *Anasa* nor *Diabrotica* were there any numerical differences between the gonial and the somatic chromosomes, such as have been described in the rabbit by Winiwarther, and more recently, in man by Wieman, in *Osmia cornuta* by Armbruster, and in *Diaptomus* by Krimmel.

LITERATURE CITED.

Armbruster, L.

'13 Chromosomenverhältnisse bei der Spermatogenese solitärer *Apiden*. Arch. f. Zellf., Bd. 11, H. 2.

Krimmel, O.

'10 Chromosomenverhältnisse in generativen und somatischen Mitosen bei *Diaptomus coerulens* etc. Zool. Anz., Bd. 35.

Morrill, C. V.

'10 The Chromosomes in the Oögenesis, Fertilization and Cleavage of Coreid Hemiptera. BIOL. BULL., Vol. XIX., No. 2.

Stevens, N. M.

'07 The Chromosomes in *Diabrotica vittata*, *Diabrotica soror*, and *Diabrotica 12-punctata*. Jour. Exp. Zool., Vol. 5.

Wieman, H. L.

'13 Chromosomes in Man. Am. Journ. Anat., Vol. 14, No. 4.

Wilson, E. B.

'06 Studies on Chromosomes. III. Journ. Exp. Zool., Vol. 3.

Winiwarther, H. von.

'00 Le corpuscule intermediaire et le nombre des chromosomes du Lapin. Arch. d. Biol., T. 16.

THE ECOLOGICAL IMPORTANCE OF THE RHEOTACTIC REACTION OF STREAM ISOPODS.

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Earlier Work on Rheotaxis in Isopods.

Some time ago it was shown that the stream mores of the isopod *Asellus communis* (Say) is usually positive in its rheotactic reaction ('12).[1] When measured in an intermittent circular current[2] this was shown by the relatively high percentage of positive responses (79 per cent. of 1,570 trials). In a continuous straight current the same tendency was shown by the relatively long time (7 hrs., 12 min.) which stream isopods would remain positive before their ultimate reversal. Pond isopods of the same species, on the other hand, gave weak positive reaction in the intermittent current (25 per cent. of 775 trials) and there was an average of only 51 minutes of positive reaction before reversal in a continuous straight current.

The difference in the reaction of the isopods from the two habitats is closely correlated with certain natural conditions, the most important of which is the oxygen content of the water. The oxygen tension of the streams which isopods inhabit is much higher than that of the ponds. Laboratory tests show that increasing the amount of oxygen present in water makes pond isopods more positive and decreasing the oxygen tension tends to make stream isopods less positively rheotactic.

But even under uniform and favorable external conditions not all stream isopods are positive all the time and some individuals show more daily variations than others ('13*a*). These variations have been analyzed by means of the resistance of isopods to potassium cyanide ('14) and by the rate of carbon dioxide production accompanying different degrees of positive rheotactic reactions (Allee and Tashiro, '14). These analyses indicate clearly that the degree of positiveness of the rheotactic reaction of an isopod is closely correlated with its rate of metabolic activity; that a highly positive rheotactic reaction is correlated with a relatively rapid rate of metabolic activity and that when either internal or external conditions interfere with the metabolic rate of the isopod, the rheotactic reaction becomes less positive.

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[1] Numerals standing alone have reference to earlier papers by the author.

[2] For details of the method see Allee, '12, p. 276; '13, p. 261.

There are certain periods in the life cycle of stream isopods when the positiveness of the rheotactic reaction is markedly reduced. These may or may not be due to the metabolic conditions obtaining at the time. The young isopod either disregards or is very erratic in its response to a water current until it is about a month old ('12). Later at the frequently recurring molting periods the same tendency is shown for about five hours preceding and following the molt ('13, '13*a*). The breeding period is also a period of weakened positive rheotactic response ('12) in which the stream isopods lose about two-thirds of their positiveness.

The breeding season begins rather later in the stream than in the pond mores ('11) but it is in full swing in April and early May. By late May the adults become rare but the season's young may be taken one third or more grown. These young isopods are at first indefinite in their rheotactic reactions. They become highly positive more rapidly in water which has a high oxygen tension but they will come to be highly positively rheotactic even if confined to pools that have a low oxygen content ('12).

SURVEY OF STREAM DISTRIBUTION OF *Asellus communis* IN THE CHICAGO AREA.

I have studied the local distribution of *Asellus communis* in the Chicago area only (Shelford, '11, '13, maps). Most of the stream isopods used in the experimental studies summarized above were taken from the County Line Creek, which flows into Lake Michigan near Glencoe, Illinois. About three sixteenths of a mile above its mouth the creek branches and its total length, including tributaries, is about half a mile. This is a physiographically young stream in the intermittent rapids, permanent pool stage with numerous stones scattered over its bed. It is ranked by Shelford ('13) as the third in age of his series of twenty-six stations in the study of stream ecology.

Stream isopods also occur in the following streams:

1. Pettibone Creek, a large stream north of the County Line Creek. Shelford ranks this as next older than the County Line Creek in his physiographic series. In many ways it resembles the younger stream but is more permanent and contains less sewage. The isopods are much less abundant.

2. North Branch of the Chicago River at Des Plaines, Illinois. Shelford ranks this as a "moderately swift medium-sized stream" and as station 16 in his stream series of 26 stations. When visited in August, 1911, the stream was reduced to a series of pools without connection, hence the part visited was younger than Thorn Creek. The isopods were found among the numerous stones on the bottom of the pools.

3. Thorn Creek at Thornton, Illinois. Shelford lists this as station 12 in his stream series. This is a rapid permanent stream about 20 feet wide at ordinary flow. There is much sewage present from Chicago Heights. The isopods here are scarce, being confined to (*a*) protruding roots under overhanging banks; (*b*) under the edge of rocks in the wide shallow riffles; (*c*) in embayments or among relatively permanent catches of drift. This stream represents about the largest, oldest stream in which the stream mores of these isopods can maintain themselves. Isopods may occur in very old streams of the sluggish base-level type, as in the Fox River at Cary, Illinois, but the part of the stream in which they are found is ecologically equivalent to a mature pond (Station 35, p. 34; Shelford '13).

From this study of the streams which contain isopods it was found that the stream mores of *Asellus communis* are most numerous in young streams of the intermittent rapids, permanent pool type; especially those that contain some sewage, and that they become more rare as the streams become larger until they are entirely lacking in many streams of the Thorn Creek stage of development. Also they are not found in streams that do not afford a good chance for lodgment.

The distribution of isopods shows a decided seasonal variation in the County Line Creek which is the only one of the streams listed above which has been sufficiently studied to give conclusive evidence on this point. In the dryer periods they are of course limited to the pools. When the stream is flowing they tend to scatter over the bottom among the debris or under overhanging banks where they may congregate in large numbers clinging to protruding roots. During the summer period the isopods are often collected in large groups along the edge of the masses of leaves that have been gathered and forced together by the spring floods. During late summer and early autumn they may be easily collected by finding and securing these groups. Later in the autumn the isopods tend to occupy the whole stream, crawling over the bottom and among the new fallen leaves. In the spring they are still more widely scattered.

Relation of Rheotaxis to Maintenance of Position in Streams.

How much of this seasonal and regional distribution is determined by the rheotactic reaction *per se?*

In 1908 the entire County Line Creek was dry except the pool nearest the lake. The rainfall of the following spring was normal. Shelford ('11) found that under these conditions the fish had all been driven to the lowest pool and in 1909 only one species, the horned dace (*Semotilus atromaculatus*) had made any progress upstream and that only about three rods. In July, 1909, the isopod *A. communis*, was found in the second permanent pool from the source and was abundant a quarter of a mile from the lake.

The horned dace is much more efficient in its rheotactic reaction than is *Asellus*, hence this distribution further towards the headwaters of a stream that was dry the preceding year must must be accounted for by some other factor than rheotaxis. Stream isopods if placed on a dirt bottom in a pan of water that is allowed to dry, will burrow into the moist mud and thus escape death for some time and it is highly probable that they were able to survive the drouth of 1908 in this manner and to take up their usual places though in less than their usual numbers with the late autumn rains.

Another line of evidence that tends to minimize the importance of rheotactic reaction in the ecology of stream isopods is found in the analysis of the reaction itself. In 6,630 discontinuous minute reaction periods 95 per cent. of which resulted in positive rheotactic responses the average rate of movement was 76 cm. per minute. The current in which these trials were made averaged 472.5 cm. per minute, hence this represents the response to a mild current. When exposed to the continuous action of a straight current of about this strength the most positive stream isopods reversed their reaction and became negative in less than twenty-four hours. As the rate of the current was increased in laboratory tests the isopods came to rest and clung to the bottom, making no effort to advance. This reaction was given before the current became as strong as that found in parts of the County Line Creek.

These observations show that positive rheotaxis may enable isopods to maintain their position for a time in parts of a stream having a weak current but after continued exposure or in strong currents the clinging reaction becomes the more important one. This is in accord with the fact that isopods are only found in streams having abundant places for support (p. 55). If the clinging reaction is essential for the most positively rheotactic adults, it must be much more so for the early juvenile stages and for adults in the breeding season or during the molting period when the positive rheotactic reaction is either weakened or entirely absent.

There is no correlation between the seasonal variation in the positiveness of the rheotactic reaction and the seasonal distribution, as described for the County Line Creek, but the distribution can be fully accounted for by the seasonal variation in places of lodgment and surfaces that give a good foothold for crawling, that is to say in the seasonal changes in the distribution of leaves in the stream. Thus in seasonal as in regional distribution thigmotaxis seems to be of primary importance rather than rheotaxis.

Interrelations of Thigmotaxis and Rheotaxis.

In testing for interrelations between the rheotactic and thigmotactic reactions of isopods that might prove of ecological importance the following methods were employed: Isopods were tested individually in an intermittent circular current for their rheotactic reaction; immediately following this the individuals were tested in a straight trough to find how strong a water current they could resist.

These tests were made in a wooden trough painted with water proof paint over which fine quartz sand had been sprinkled while the paint was soft in order to give a good surface for the isopods. The sides met at the bottom forming a V-shaped trough with an enclosed angle of about thirty degrees. The trough was 75 cm. long and was used with a pitch of 8 cm. The current was produced by tap water.

In making the test the isopod was oriented as desired in a very small stream of water. A rubber tube 5 mm. in diameter connected with the tap faucet was introduced 5 cm. above the isopod. The faucet valve was gradually opened until the current swept the isopod off its feet. This occurred in less than a minute so that fatigue did not affect the result. Then the flow in cubic centimeters was measured. In the early work five successive tests were averaged to give the resistance of the isopod for the day but this was found to be too severe and in the later work only one test was made per day.

In the weak current the isopods would usually start to move in the direction in which they were headed ('12) although those oriented negatively attempted to turn more often than the others. They usually continued moving until the current became fairly strong, then stopped and clung to the boards in the angle at the bottom of the trough.

When headed upstream the head was placed close to the bottom as movement ceased the head and front of the thorax made an arch which kept the water running over the relatively smooth convex back of the isopod. The anterior legs are used more in clinging than the posterior ones, which act as braces. When thus clamped the isopods withstood a flow as high as 6,600 c.c. per minute under the conditions of the experiment. Sometimes they failed to clamp when headed positively but this occurred less often than in any other position. The speed of the current successfully resisted appeared to fully equal or surpass the most rapid flow at usual stream levels in those streams in which the isopods are abundant.

When oriented negatively the isopods may clamp and withstand a strong current (up to 3,959 c.c. per minute as compared with 6,600 c.c. per minute when oriented positively). In this reaction the abdomen is bent downward much as is the head when the animal is turned in the opposite direction, but it is more easily forced up than the head and hence this is a less efficient method for clinging. Isopods fail to clamp more often under these conditions than when headed positively. This may be due to the difference in length of the posterior and anterior legs. The latter are short and can take more active part in the clamping reaction than can the longer posterior ones. The failure to clamp allows the negatively oriented isopods to be more often swept off their feet by a relatively slow current.

Comparative tests of the average clinging efficiency of isopods oriented positively and negatively support the evidence given by the maximum clinging ability under the two conditions. The results of these tests which were made under identical conditions are given in Table I. The orientation of the isopod was alternated to equalize a possible fatigue effect. In every case except isopod No. 170 the strength of the current resisted when the isopods were headed upstream was approximately double that necessary to sweep the negatively headed isopods off their feet. An average of 64 tests on 10 different isopods shows that a flow of 2,534 (±72) c.c. per minute was required to sweep a positively oriented isopod off its feet while 1,385 (±92.6) caused those headed negatively to lose their footing. In other words a positively oriented isopod is approximately twice as efficient in maintaining its position in a stream as is one oriented negatively.

There are indications that isopods could maintain themselves

TABLE I.

Showing the effect of orientation upon the strength of current in which isopods can maintain their position. These tests were made under entirely identical conditions throughout. In testing each animal, one to three trials were made with the isopod facing in one direction then the orientation was reversed and an equal number of trials were made. This was done to equalize possible fatigue effects. For further details of the method used see p. 57.

Oriented Positively.

Trials.	Identification No.									
	168	169	170	171[1]	172	21	22	23	168	173
1	4,750	1,300	2,750	2,750	4,350	3,000	2,100	1,140	4,033	3,450
2	3,350	2,400	2,000	2,500	2,850	2,160	1,800	1,500	2,200	2,100
3	4,000	3,400	1,800	2,000	3,850	1,500	2,100	1,620	2,550	3,450
4		1,500	3,750	2,700	3,300	2,280	2,280	2,580	2,490	3,300
5		2,400	2,350	2,500	3,300	1,560	5,100	2,820	3,530	3,900
6						540	1,800	3,000	3,240	
7							2,000	2,280		
8							2,160	1,950		
9							1,800	1,800		
10							2,160	1,560		
11							2,280	2,400		
12							900	1,920		
Average .	4,033	2,200	2,550	2,490	3,530	1,973	2,207	2,072	3,007	3,240

General average of 64 trials, 2,534 (± 72)

Oriented Negatively.

Trials.	Identification No.									
	168	169	170	171[1]	172	21	22	23	168	173
1	3,300	600	3,050	850	200	1,500	2,160	1,440	1,800	200
2	1,900	600	2,500	450	300	2,040	600	1,380	1,360	200
3	200	1,400	3,950	1,900	350	1,860	360	300	2,610	200
4		2,500	1,800	1,050	2,700	1,080	420	1,200	850	2,800
5		1,700	2,650	2,750	3,600	2,280	1,920	1,260	1,430	2,800
6						1,920	2,760	360	1,240	
7							300	1,080		
8							1,920	600		
9							600	1,800		
10							300	2,520		
11							300	1,760		
12							200	600		
Average .	1,800	1,360	2,610	850	1,430	1,780	987	1,192	1,547	1,240

General average of 64 trials, 1385 (± 82).

in quite strong currents when headed at right angles to them. In doing this an isopod used the feet of one side as anchors and swung below them. This method of maintenance gave erratic results with the apparatus used. It also brought the isopod into different relations with the current since when oriented transversely part of the body would be near the surface instead of the whole body being at the bottom, as is the case when the animals are oriented either positively or negatively. Since with the present apparatus it was impossible to obtain comparable results and since stream isopods usually go either positive or negative this method of maintenance will not be discussed further.

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[1] Copulating pair.

The data so far presented make it evident that the rheotactic reaction *per se* is not sufficient to account for the maintenance of position of stream isopods but that it has an effect upon the clinging ability of isopods in the current that is very important. In other words the movement accomplished in the rheotactic reaction of isopods is of much less importance in maintaining their position in streams than is the sign of the rheotactic reaction, which becomes of prime importance by means of its influence upon the clinging ability of isopods. Since the sign of the rheotactic reaction is closely correlated with the metabolic rate of isopods it follows that an isopod with a relatively high rate of metabolism would give positive rheotactic responses and hence be better able to maintain its position in a stream than an isopod having a relatively low rate of metabolism which would be accompanied by negative or indefinite rheotactic reactions.

It has already been noted that there are two periods in the natural life of adult stream isopods when the usual highly positive rheotactic reaction is lowered, namely: the breeding season and the shorter but more frequently recurring molting period. Both of these times are the more critical in that the clinging ability is, or may be, directly affected by them. During and immediately after molting an isopod is easily swept off its feet by a water current and in the breeding season at the time of copulation the pairs have their clinging ability reduced, especially when oriented negatively (cf. No. 171, in Table I., p. 59). After the female liberates the young and before the brood pouch is molted, a bubble of air may get into it and an isopod thus afflicted finds it almost impossible to remain on the bottom even in quiet water. These floaters often occur in the laboratory and have been observed to some extent in nature. Thus both the molting period and the breeding season reduce the maintenance efficiency of stream isopods, directly by decreasing the clinging ability and indirectly by decreasing tendency to orient positively in the current which in turn affects the clinging ability of the isopod.

The rheotactic reaction has been shown to vary with the changing metabolic state of the isopod (Allee and Tashiro, '14). The clinging power of positively oriented isopods also varies to a limited extent, with the degree of positiveness of the rheotactic reaction. This is shown by the data exhibited in Tables II. and III. In Table II, the average rheotactic response of isopods that resisted a current of 3,500 c.c. per minute or more is compared with that given by isopods that were swept off their feet by a current of 2,000 c.c. per minute or less. The isopods with the higher clinging power averaged 78 per cent. positive in their rheotactic reactions and they did not fail to give the rheotactic response when stimulated. On the other hand the isopods with the poorer clinging power averaged 57 per cent.

Table II.

Showing the relation between low and high clinging ability of positively oriented isopods and the rheotactic reaction. For details of methods see pp. 57 and 61.

Responses Compared.	No. Trials.	Ave. Current Resisted.	Average Rheotactic Reaction.				Ave. Efficiency.[3]
			+	—	a	o	
Current resisted 3,500 c.c. per minute or more	65	4,418	78 (±2.6)	18	4	0	2.7
Current resisted, 2,000 c.c. per minute or less	99	1,497	57 (±2.6)	23	10	10	2.3

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[3] In order to have a method for measuring and recording the efficiency of movement of isopods in the current during rheotactic tests the following arbitrary standard was adopted, which represents numerically the distance covered in a minute's reaction period. The following values were applied to the numbers:

0, no reaction.
1, slight movement.
2, any response between 1 and 3.
3, progress one third around the pan positively (approximately 27 cm.) or two thirds, negative (54 cm.).
4, progress two thirds around the pan positive, or one and one third negative.
5, progress once around the pan positive, or twice around negative.
6, any distance over 5.

Experiments showed that isopods oriented negatively covered twice the distance per leg movement as did those oriented positively.

positive and gave no response to the current in 10 per cent. of the trials. Thus the rheotactic reaction averaged 21 per cent. less positive when the isopods had the lower clinging power. Since this is four times the probable error it must be accepted as strongly indicating that isopods with high clinging ability when positively oriented, tend to give a higher percentage of positive rheotactic reactions than those with low clinging power.

The converse data of this relationship is shown in Table III,. which lists the average current strength necessary to sweep isopods off their feet that had given: (*a*) highly positive rheotactic reactions; (*b*) low positive rheotactic reactions; (*c*) a high percentage of failures to respond to the current. In all cases the only variation known was in the metabolic state of the isopods.

Table III.

Showing the relation between clinging ability of positively oriented isopods accompanying a rheotactic reaction of 60 per cent. or more positive; 40 per cent. or less positive: and 40 per cent. or more no reaction. For details of method see pp. 57 and 62.

Responses Compared.	No. Trials.	Ave. Rheotactic Reaction.				Ave. Efficiency.[4]	Ave. Current Resisted.
		+	—	*a*	o		
Rheotaxis 60 per cent. or more positive	160	90	6	4	0	2.7	2,898 (±66)
Rheotaxis 40 per cent. or less positive	87	13	45	22	20	2.1	2,230 (±81)
Rheotaxis 40 per cent. or more no reaction	21	5	1	15	79	0.24	1,990 (±109.5)

The data presented in Table III., show that isopods having a 13 per cent. positive rheotactic reaction gave 23 per cent. less clinging ability than those that were 90 per cent. positive in their rheotactic reactions. With isopods that gave a high degree of inaction in the rheotactic tests there was a further reduction in the clinging power. The change in the strength of current successfully resisted is not at all proportional to the variation in the rheotactic reaction with which it is compared but the difference in clinging power accompanying high and low positiveness of the rheotactic reaction is 4.5 times the probable error and is therefore significant.

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[4] For explanation of average efficiency see footnote 3, p. 61.

Not enough data has been collected to warrant any conclusions being drawn regarding the possible relationship between the clinging ability of isopods and their metabolic rate as measured either by their resistance to potassium cyanide or their carbon dioxide production. However the data given in Tables II. and III. show that there is a variation in the clinging power of positively oriented isopods accompanying more or less closely the variation in the positiveness of the rheotactic reaction. Since the degree of positiveness of this reaction depends on the rate of metabolism (*loc. cit.*) it seems fair to conclude that there is some correlation between the physiological states of isopods and their ability to maintain themselves in a water current. This means that a decrease in the metabolic activities of isopods not only causes a decrease in the positiveness of the rheotactic reactions and hence indirectly decreases the clinging power of isopods in a current (p. 60) but that it also directly decreases the clinging power.

Discussion.

In all probability the sum total of the reactions of an organism largely determines its distribution (Shelford and Allee, '13; Wells, '14). With different animals in different environments now one reaction or group of reactions may be the more important and now another. It is obvious that the maintenance of position is of primary importance in the distribution of stream animals and it is likewise obvious that this must be done either by some means of clinging or by the rheotactic reaction or by a combination of the two. Bryozoa show the maximum development of the clinging reaction while certain fishes as *Notropis* show a maximum development of the rheotactic response since they rarely rest on the bottom (Shelford and Allee, '13). A study of maintenance methods of stream animals would probably establish a completely graded series between these two extremes. In such a graded series all crawling animals, to which group the isopods belong, would of necessity depend a great deal upon their ability of clinging to the substratum to maintain themselves.

To what extent positive orientation in the rheotactic response increases the clinging efficiency of crawling stream animals other than isopods is a matter for experimentation. Judging solely from structure it would seem entirely probable that such long bodied animals as *Hydropsyche* larvæ, dragon-fly or may-fly nymphs and stone-fly nymphs would show this correlation to some extent. Among fishes, the bottom resting darting fishes of the type represented by *Boleosoma* and *Etheostoma* should also show this correlation. Further experimentation on these forms would probably demonstrate, as the experiments here reported for isopods, that the sign of the rheotactic reaction continues to be of prime importance in the problem of maintenance of position in streams long after the rheotactic movements have ceased to be significant.

How much of this behavior of stream isopods is an adaptation to life in streams? In order to get the problem clearly stated perhaps a recapitulation of relationships is permissible. By means of their highly positive rheotactic reactions the stream isopods are better enabled to maintain themselves in the stream environment through the interaction of the rheotactic and thigmotactic reactions. This tendency to give positive rheotactic reactions is much weaker in pond isopods and can be modified within certain bounds in both pond and stream mores by varying the oxygen tension of the water. Therefore it seems that this is not a specific adaptation for stream habitation which the isopods exhibit, but rather that adaptation (perhaps better called property), of all living matter namely, irritability (Mathews, '13). Beyond that, the isopod is no more adapted to the stream environment by reason of its positive rheotaxis than the stream is adapted to the isopod by virtue of presenting the complex of environmental conditions (high oxygen and low carbon dioxide tension) best calculated to call forth and maintain positive rheotaxis. Environmental conditions automatically cause an isopod to give those responses which fit it to maintain its position in the stream.

Summary.

1. The distribution of the isopod *Asellus communis* cannot be accounted for by its rheotactic reaction alone, but can be accounted for by the interacting thigmotactic and rheotactic reactions.

2. The movements given in rheotactic reactions of isopods are

of secondary importance in their maintenance of position in streams.

3. The sign of the rheotactic reaction is of primary importance in this regard in that an isopod oriented positively can withstand approximately twice the current strength of one oriented in the opposite direction.

4. The clinging ability of positively oriented isopods varies somewhat with the degree of positiveness of the rheotactic reaction which in turn is closely correlated with the metabolic conditions of the isopods.

5. The molting period and breeding season are especially important in the ecology of stream isopods in that they directly decrease the clinging ability and indirectly affect it by lowering the tendency to give positive rheotactic reactions.

6. Beyond possessing irritable protoplasm isopods are no more adapted to the stream environment by reason of their positive rheotaxis than the stream is adapted to the isopod by virtue of presenting the complex of environmental conditions best calculated to call forth and maintain positive rheotaxis.

LITERATURE CITED.

Allee, W. C.

'11 Seasonal Succession in Old Forest Ponds. Trans. Ill. Acad. Sci., Vol. 4, pp. 126–131.

'12 An Experimental Analysis of the Relation Between Physiological States and Rheotaxis in Isopoda. Journ. Exp. Zoöl., Vol. 13, pp. 269–344.

'13 The Effect of Molting on Rheotaxis in Isopods. Science, N.S., Vol. 37, pp. 882–3.

'13a Further Studies on Physiological States and Rheotaxis in Isopoda. Journ. Exp. Zoöl., Vol. 15, pp. 257–295.

'14 Certain Relations between Rheotaxis in Isopods and their Survival Time in Potassium Cyanide. Journ. Exp. Zoöl., Vol. 16, pp. 397–412.

Allee, W. C. and Tashiro, Shiro.

'14 Some Relations between Rheotaxis and the Rate of Carbon Dioxide Production of Isopods. Journ. An. Beh., Vol. 4, pp. 202–214.

Mathews, A. P.

'13 Adaptation from the Point of View of the Physiologist. Am. Nat., Vol. 47, pp. 90–105.

Henderson, L. J.

'13 The Fitness of the Environment. An Inquiry into the Biological Fitness of the Environment. MacMillan, New York, 317 pp.

Shelford, V. E.

'11 Ecological Succession, I. Stream Fishes and the Method of Physiographic Analysis. BIOL. BULL., Vol. 21, pp. 9–36.

'13 Animal Communities in Temperate America. Univ. of Chicago press. 362 pp.

Shelford, V. E. and Allee, W. C.

'13 The Reactions of Fishes to Gradients of Dissolved Atmospheric Gases. Journ. Exp. Zoöl., Vol. 14, pp. 207–266.

Wells, Morris M.

'13 The Resistance of Fishes to Different Concentrations and Combinations of Oxygen and Carbon Dioxide. Biol. Bull., Vol. 25, pp. 323–347.

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BIOLOGICAL BULLETIN

THE EFFECT OF X-RAYS ON THE RATE OF CELL DIVISION IN THE EARLY CLEAVAGE OF PLANORBIS.[1]

A. RICHARDS.

Only a very short time had elapsed after the announcements of the discovery of X-rays (in 1895) and of radium (in 1898), when it became generally known that animal and plant life can be profoundly affected by radioactivity. Subsequently, a large amount of experimentation has been done and many interesting results prove the powerful action of these agents upon living matter. Physiological and therapeutic studies of radioactivity have long since given a firm empirical foundation for its application in the cure of disease. From the pure biological standpoint, also, experimentation has not been lacking; instead, a long list of titles stands to its credit.

However, most of this purely biological work has concerned itself with the production of abnormalities either in the embryo or in the adult. Only recently has there been any attempt to analyze these results from the study of the units which make up the tissues affected. Nevertheless, it seems clear that the effects upon an organism of radioactivity, or of any agent which produces abnormalities, must depend very largely for any real explanation on results obtained from the study of the effects of that agent upon the cellular elements making up the organism. In the present cases the character of the animals studied accounts for the lack of data at hand upon the more detailed effects on the cells of the tissues in question. Generally vertebrates have been chosen as subjects of experiment and observation and their cells with a few exceptions have not proven suitable for a study of cytological details.

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[1] Contribution from the Zoological Laboratory of the University of Texas, No 117.

With the idea of finding more exactly what occurs to individual cells when exposed to X-rays, the writer studied the eggs of the freshwater snail, *Planorbis lentus*, in relation to this problem. By choosing such a form, several advantages are gained. The eggs divide in a very definite manner and the normal course of their development has been carefully observed, as has that of many related gasteropods. It is, therefore, a comparatively simple matter to study at least the more gross effects of the exposures, and to compare with experiments of most varied character upon forms not dissimilar in the details of their development to the one here employed.

Furthermore, there is hope that the use of radioactivity in experiments on eggs of well-known type may lead to further knowledge of the principles of egg structure and organization. The reactions which the eggs give to exposure to X-rays must be, if constant, the expression of some quite definite mechanism within the egg to which the X-rays act as a stimulus. A comparison of these reactions with the responses of this mechanism to other stimuli of different nature very possibly may lead to interesting conclusions as to the nature of the mechanism itself. This, of course, is the much broader biological problem.

In most of the work which has been done recently from the standpoint of pure biology radium has been the agent used for experimentation. In general one would expect that the results obtained from radium rays would be similar to those from X-rays; but it is not possible to predict that such is the case and the results with radium have been comparatively meager. Radium rays are of three kinds, α, β, and γ; of these the γ rays are the more penetrating and to them are probably due most of the effects on living forms. From comparative studies made by physicists it is well known that the γ rays of radium are quite similar in many particulars to the X-rays, and it is stated by Rutherford that they are in fact the more penetrating X-rays. In view of the facts, therefore, that it is perhaps easier to understand something of the nature of the disturbances caused by the X-rays, and that this form of radio-activity is more easily obtainable at this laboratory, it was determined to use X-rays rather than radium. No difficulty has been experienced in getting results with the X-rays.

The work which is here reported was carried on at the University of Texas and at the laboratory of the U. S. Bureau of Fisheries at Woods Hole, Mass. The Bureau of Fisheries has kindly given permission for the publication of results obtained at its laboratory.

For the use of the X-ray machine, which he kindly loaned me, and for his assistance in various ways during the early course of these experiments, I am indebted to Mr. Oliver Brush, of Austin, Texas.

The snails used were identified for me as *Planorbis lentus* Say by Dr. W. H. Dall, of the Smithsonian Institution, to whom I wish to express my obligation. They were secured from Waller Creek, a small stream near the University of Texas.

These experiments were conducted during the early part of the year 1913. After the results had been studied and written up, it appeared wise to delay publication until another breeding season could furnish new material and further experiments could be carried on in order to extend the observations and perhaps give rise to broader conclusions. During December, 1913, however, Texas was visited by one of the most severe floods in its history and the streams were cleanly scoured out. Conditions of vegetation also were greatly changed. As a result, where formerly *Planorbis* had been found most numerously during the spring months, there are now only a very few scattered specimens. Furthermore, much difficulty has been experienced in getting these specimens to produce eggs. For these reasons I have been unable to renew the experiments on *Planorbis*. Other fresh water snails suffered largely the same fate during the flood, but their pointed shape enabled more of them to maintain themselves against it, and I have been able, therefore, to study somewhat the effects of the rays on *Physa halei*. As these eggs are less suitable for detailed study in the living condition owing to the thickness of their gelatinous covering, the statements made in this account apply chiefly to the eggs of *Planorbis*. In general, the behavior under exposure of the eggs of these two species has not been found to differ much. Reference to these more recent observations will be made in the appropriate places under the later discussion.

METHODS.

Specimens of *Planorbis* may be kept in aquaria, and during the night they will lay on the sides of the glass dishes or on the water plants that may be in the aquaria. If lily pads are placed in the aquaria, their rough lower surfaces seem to be favorable places for finding clusters of these eggs. The eggs occur in "clusters" (Holmes) which are bound together by tough enclosing membranes and which contain a considerable amount of jelly. Within the clusters there are, perhaps, a couple of dozen capsules filled with yellow albumen mass in which the *Planorbis* egg itself develops.[1]

The eggs of *Planorbis* are mostly laid at night, or usually just before day. Observations made early in the morning, say 7 A.M., frequently find eggs which have not yet put out the first polar body.

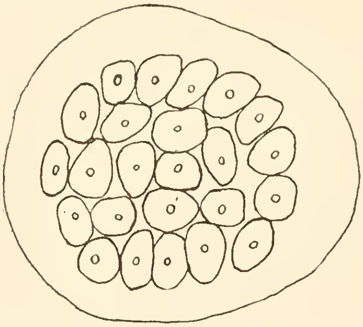

FIG. 1. Egg cluster of *Planorbis*, showing eggs within the albumen capsules, all of which are surrounded by a gelatinous mass. (From Holmes.)

In studying the eggs of *Planorbis*, the general method of procedure has been as follows: the clusters of eggs have been removed from the sides of the glass dish or from the plants upon which they were deposited by inserting a sharp knife between them and the attachment, care being taken not to break the gelatinous capsules. The eggs can now be studied under conditions which do not differ widely from the normal by merely placing them in a watch glass, and observing them under a microscope. To expose the eggs to X-rays it is only necessary to set the watch glass under the X-ray tube.

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[1] See Holmes's description, *Jour. Morph.*, Vol. 16.

When considering the results of these experiments and the effects obtained on *Planorbis* eggs it must be kept in mind that these eggs are normally subjected to wide variation in temperature—from mild winter temperature to summer heat—and they are unusually well protected. The description given by Holmes (p. 375) of the unsegmented eggs of *Planorbis trivolvis* fits the egg of *Planorbis lentus* accurately. He describes the egg as embedded in a thick albumen mass within a capsule, which in turn is surrounded by a jelly mass, and the whole including a score of other similar capsules, is covered by a tough enclosing membrane. In making the experiment the entire cluster only is disturbed. Thus there are eliminated from consideration such factors as changes of temperature, of pressure, of oxygen or carbon dioxide content, etc. In fact the eggs were developing under entirely normal conditions, with the exception that the cluster had been freed from its attachment, at the time of exposure. The only factors that this loosening and the subsequent treatment of the eggs could involve, except the factor under experiment, would be a very slight degree of shaking, changes in the direction of the attraction of gravitation, and changes in light intensity; and it is highly improbable that either of these would have much effect upon the rate of development, or upon the finer structure of the egg.

When it is desired to fix the eggs it is only necessary to break the capsule with a needle and tease them out. I have teased them as Holmes recommends into salt solution which contained a little picric acid and then fixed them in Kleinenberg's picro-sulphuric.

Under proper conditions of illumination, it is usually possible to observe the grosser details of spindle and aster formation in the living egg, and the early cleavages are not difficult to follow. The normal course of cleavage is approximately as described by Holmes ('00) for *Planorbis trivolvis* and by Conklin for *Crepidula* ('97). The cytological details as worked out on the latter animal (Conklin, '02) apply to a large extent to the development of *Planorbis*.

The progress of the division as seen in the living egg is briefly as follows. The nucleus can be seen in the resting state as a clear spherical area distinguishable from the rest of the cytoplasm by its lighter color. As the spindle progresses the egg elongates slightly in the axis of the spindle in preparation for division. In the succeeding cleavage this elongation is more clearly marked.

After the furrow has separated the blastomeres, they round up until they are almost spherical in form and the contact surface forms only a narrow protoplasmic bridge between them. This rounding off of the blastomeres is repeated after each cleavage, at least after the earlier cleavages, and suggests, as Holmes points out, the result of some tension which is exercised at the poles of the eggs. The tension soon appears to decrease, however, for the blastomeres flatten against each other gradually as if they were being drawn together, with the result that each assumes a more or less spherical form and the contact surface becomes a mere line between the eggs. This flattening is accomplished by a rotation of the blastomeres toward the animal pole. The nuclei and the asters thus come to lie near the animal pole and the spindle is bent sharply (cf. Conklin, '02). Presently, after the blastomeres have flattened against each other in the two-cell stage, a "lenticular cleavage cavity" (Holmes) appears between them, and its maximum is a criterion as to the progress of the next stage.

Since these stages occur with little variation and can be seen rather clearly in the living condition, it is possible to expose the egg to the X-rays at almost any time desired.

During the resting stage the nuclei when viewed from the animal pole lie very near the furrow. Thus the two nuclei of the separate blastomeres are brought as close to each other as possible. At a little later stage the nucleus in each blastomere passes down toward the center.

The cytoplasmic constriction cuts in deeper at the animal pole on the first cleavage as Holmes has pointed out; this is the general rule with eggs of this type. It holds true for the succeeding cleavages, but in cases of some eggs which have been exposed to the X-rays I have noticed that the second furrow is the one to cut in more deeply.

When the nuclei of the blastomeres have moved to the center they begin the second division. By the time the cytoplasmic division can be seen the nuclear division is well under way. It is the rule to which exceptions are only occasionally seen that the two cells should divide at the same time. However, it is true that one cell will sometimes divide before the other has progressed far, and a three-cell condition results as shown in Plate I., Fig. 8, of Conklin's ('97) paper.

The blastomeres again elongate in the direction of the long axis of the spindle. The furrow begins to cut in from the sides and from the animal poles. The cells again round off after the division, and we again find the cleavage cavity present. It differs from the first cleavage cavity, however; in addition to the lenticular spaces which appear between each pair of dividing cells, a large rectangular cavity is to be seen at the center.

The third cleavage consists in the giving off of the first quartette of micromeres (ectomeres) at the animal pole. A spiral dextrotropic shifting of the quartette occurs after which the cells flatten; and there remains once more an almost spherical mass of cells. A central cleavage cavity again makes its appearance and again disappears after the next cleavage.

The four macromeres divide now to produce a second quartette of micromeres; thus arises the twelve-cell stage. As before, after the division the cells flatten to form a spherical mass. In the living condition it is most easy to determine which stage is being observed when one can see the egg in an optical section running from pole to pole. The division of the first quartette of ectomeres now produces the sixteen-cell stage which in turn give rise to the twenty-four-cell stage by division of the second quartette of ectomeres and at the same time a division of the macromeres by which the third quartette of ectomeres is given off. According to Holmes "the twenty-four-cell stage, which is reached by these divisions, marks a resting stage of considerable length in the development of the egg. A cleavage cavity is formed at this time which may acquire quite a large size" (page 380). This is the stage at which in general observations on the living eggs as to the effect of exposure to the X-rays was closed.

To sum up: the first division produces the cells *AB*, and *CD;* the second division *A*, *B*, *C*, and *D;* the third division 1*A*, 1*a;* the next, which is the 12-cell stage, consists of 2*A*, 1*a*, 2*a;* the 16-cell stage consists of 2*A*, $1a^1$, $1a^2$, 2*a;* the 24-cell stage consists of 3*A*, $1a^1$, $1a^2$, $2a^1$, $2a^2$, and 3*a*.

Experiments and Observations.

Eggs of *Planorbis* were exposed to the X-rays while immersed in water in a watch glass. First they were carefully observed under a microscope and the degree of their development noted. The glass containing them was then placed at a distance of about four inches below a tube of average hardness (after the current had been started through the tube). An automatic regulating device on the tube used made possible a fairly uniform exposure. At the close of the exposure the eggs were again examined and the degree of development noted. Observations were then made at intervals during the day as convenience permitted. Usually at the end of five or six hours the eggs had reached a stage where further observation of them in the living condition was unprofitable. At this stage they were usually killed and fixed, although some were allowed to develop for later study.

In the cell divisions which are concerned in the maturation and cleavage of *Planorbis* eggs up to the twenty-four cell stage the writer has never observed any division under usual conditions of temperature to take place in less than forty-five minutes. It is, moreover, exceptional to find the division occurring in so short a time as this, for in general the complete cycle does not take place under an hour and often it is longer than that. Exact data for a table showing just how long a period of time elapses between each cleavage is not at hand,[1] but my records show no case in which the division was completed in less than forty-five minutes. On the other hand, cases have been observed in which fully two hours elapsed before a second cycle began.

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[1] The following figures are taken from an observation made under average conditions. They may be regarded as normal, but there is variation from this norm as stated above.

Experiment (1) The 1st cleavage division required 1½ hours for completion.
2d cleavage division required 1¼ hours for completion.
3d cleavage division required 1¼ hours for completion.
4th cleavage division required 1¼ hours for completion.
5th cleavage division required about 1 hour for completion.

It is, of course, true that the rate of division can be changed by varying temperature. Eggs which are kept in a refrigerator will require several hours to complete a division. Similarly, eggs will divide much more rapidly upon a warm day. The statements made in the preceding paragraph, however, are based upon conditions which obtain normally, or at least upon conditions which are as near normal as it is possible to come in the laboratory.

It may be conservatively stated that the range for these early cleavages is from fifty minutes to more than two hours.

If *Planorbis* eggs are exposed to the rays during the resting stage between two mitoses the results are less marked than if the exposure is during the progress of the mitosis.[1] It is quite certain from the later behavior of eggs so treated that no ex-

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Incomplete observations on *Physa* eggs seem to indicate a similar range of time values for the cleavage divisions in that form.

(This observation and those included in the succeeding footnotes form only a part of the data taken in this investigation. The arrangement here and the number of the various experiments indicate nothing more than convenience for reference. The experiments, of which there were many more than are here given, were not made in this order; these are chosen merely as examples bearing on the points under discussion.)

[1] (2) At the beginning of this exposure, the eggs had just finished the first cleavage and their nuclei were resting. They were exposed to the X-rays for six minutes. At the end of this time there were no visible effects. Thirty-five minutes later the second furrow made its appearance and in one hour and fifteen minutes after the exposure, the second division was complete. Two and one-half hours were required for the next division, a much longer time than normal.

(3) The eggs used in this experiment had been observed during the progress of the first cleavage division, and were exposed for six minutes at the time when the blastomeres were most widely separated and the nuclei were resting. The exposure had no visible effect, and the eggs apparently did not depart from their normal course. One hour and fifty minutes, however, were required for the next cleavage. The third cleavage consumed one hour and fifteen minutes, and in two hours more the sixteen cell state had been reached.

(4) At the time of exposure the first cleavage furrow had separated the blastomeres fully. The exposure lasted three minutes and produced no effect except that ten minutes after it had begun the blastomeres had flattened against each other. One hour and ten minutes after the exposure, the second furrow made its appearance in some of the eggs. Not all had progressed equally, some having nearly completed the second division. The first quartette had been given off and its division begun, at the end of one hour and forty five minutes more.

Other cases might be cited.

posure is without some effect, but whatever changes there may be induced are very slight if the exposure is during a resting stage. Only a slight stimulation of rather uncertain nature can be produced. It does not manifest itself by changing appreciably the rate of division so far as hastening is concerned; the evidence, however, would indicate that later on the phase of depression (see below) follows such an exposure although it may be in less degree. Cytological examination shows only slight effects of exposure during the resting stage on the structure of the protoplasm.

It is probably not misleading to say that during the resting stage the egg is in a state approaching equilibrium in which activation is with difficulty produced.

Recent experiments on developing eggs have in general given results similar to those just outlined. Conklin ('13) found abundant proof in his *Crepidula* experiments of the principle enunciated some years ago that dividing nuclei are more easily disturbed by environmental change than nuclei at rest. Koernicke ('05) noted that after an exposure of the roots of *Vicia Faba* and of *Pisum sativum* for two days to radium the resting nuclei appeared unaffected. This general result has been obtained so widely that it seems unnecessary to cite further proof for a position against which there is no contradictory evidence.

Following the general rule that resting nuclei are only with difficulty stimulated one would expect little result from stimulating eggs in the germinal vesicle stage. As far as effect on the rate of cell division is concerned exactly that result was obtained.[1] Fertilization had of course already occurred, for the sperm enters *Planorbis* eggs at the time of laying. The egg, therefore, at the time of exposure was beginning a new cycle of development, caused by the entrance of the sperm, but its nucleus had not yet started upon its cycle and so was not disturbed by the stimulation.

[1] (5) The eggs in this experiment were in the germinal vesicle stage at the time of the exposure, which lasted 20 seconds. At the end of the exposure no change could be noted. One hour and thirty minutes later the maturation divisions had passed and the first cleavage furrow was making its appearance. One hour later the second cleavage was nearing completion. Four and one half hours later the first quartette had divided.

(6) Eggs in germinal vesicle stage were exposed three minutes with no visible effect. Four and one half hours later they were killed and fixed in the four cell stage.

Similarly at the end of the maturation divisions before the fusion of the male and female pronuclei no marked stimulation could be produced by exposure to the X-rays.

Contrasting with the condition just described is that found in the cleavage mitosis. If one may speak of the cell in the resting nucleus stage as being in a state of equilibrium or stability with respect to its capacity to respond to stimulation, we may likewise say that during a mitosis it is relatively unstable with respect to this particular character;[1] and there appears to be a certain time during the course of spindle formation when the capacity of the cell to respond is greatest. During the period from the definite formation of the spindle to the metaphase or anaphase, response to X-ray stimulation is easiest to obtain. Similar results have been reported for experiments with various means of stimulating the egg. Conklin ('13) found much clear evidence to prove that "the early stages of cleavage are more sensitive to environmental changes than later ones."

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[1] (7) The eggs of this experiment had just completed the formation of the first polar body and the second maturation spindle was beginning to form when they were exposed to X-rays for ten minutes. At the end of the exposure the second division seemed to be entirely complete. The first cleavage division was accomplished in the next fifty minutes. The succeeding divisions occupied more time, for one hour and thirty minutes elapsed before the completion of the second cleavage, and a like period of time passed before there was any sign of a micromere division. Later divisions were even more slow. The control was far ahead in its divisions, having reached a stage where the blastomeres could no longer be counted accurately in the living condition.

(8) The eggs of this experiment were exposed ten minutes during early stages of the second cleavage spindle. When the exposure was ended it was found that in many cases the division was completed, and in all it was well along. The third division occurred in thirty five minutes more. Owing to an accident, further data on this set of eggs were not obtainable.

(9) The eggs in this set were secured during the spindle formation of the first maturation division. The exposure lasted six minutes; at its conclusion, the cytoplasm could be seen collected largely at one pole of the egg, and in some cases a polar body elevation was apparently beginning to form. In half an hour both maturation divisions were completed. One hour and twenty minutes were consumed in the first cleavage division and one hour and fifty minutes more in the second division. Two hours later no advance was noted.

(10) Eggs in the late prophase of the first cleavage were exposed three minutes, at the end of which time they were found to have completed the division, and in five minutes more the blastomeres had flattened against each other with the peculiar lenticular cavity between them. In thirty minutes the second cleavage had occured and in two hours and a half the fourth had been accomplished.

Variations in the results of exposing the eggs are probably to be explained largely on the basis of this maximum and minimum capacity for response.[1] In other words, if the exposure is made during a resting stage, the minimum stimulation results; if it is made during the period of the early spindle, the maximum stimulation is obtained; but if it is made between these two extremes, the result is neither maximal nor minimal and there is only a partial degree of stimulation, the amount depending upon the relative position of the nuclei in the mitotic cycle when the exposure took place.

The first visible effect on *Planorbis* eggs of exposure to X-rays is to stimulate their division. Any particular mitosis which may be in progress at the time of the division is hastened to a very great degree.[2] So far as may be observed in the living egg the process is not different in character from the normal indirect cell division—although the later events in the life of the egg make it certain that something essential in the mechanism has been disturbed—but the time which is consumed by a cell

[1] (11) At the time of the exposure of three minutes these eggs showed no sign of division, nor was any change noted at the end of the exposure, although in some cases, what were presumable early maturation spindles, could be seen. One hour and ten minutes later the first cleavage division had taken place, and in forty five minutes more the second furrow had appeared. In one hour from this time the first micromere division had not been completed, but in two and one quarter hours after the second furrow the second micromere quartette had been given off, making the twelve-cell stage.

Experiments (12) and (13) were exact duplicates of (11).

[2] Compare experiments (8), (10).

(14) The eggs of this set showed the first trace of the second cleavage furrow at the time of the exposure, which lasted ten minutes. At the end, the eggs were all in the four cell stage. While not all of the cluster had been exactly together at the beginning of the exposure, all apparently finished together.

(15) At the end of a six minute exposure, eggs in which the second furrow was barely visible at the beginning had now passed into the resting condition. In thirty minutes more the first micromere quartette had been completely given off. Two hours later the eggs were probably in the 16-cell stage, but it was not possible to observe exactly in this case.

(16) Eggs in the 4-cell stage were exposed three minutes. At the end of the exposure the first micromere quartette had been given off, although in a few cases not quite completely. The four macromeres were as yet spherical, not having flattened against each other; thus they gave the appearance of being almost completely separated from each other. Twenty five minutes later the second quartette had been given off, and at the end of another half hour the first had divided. One hour and thirty minutes later the egg had reached a stage comparable to Holmes' 24-cell stage, for which see Fig. 10.

undergoing mitosis at the time of the exposure is very much less than in the case of a cell dividing under more normal conditions. Thus, of the effects induced in the egg by exposure to X-rays, the first takes the form of a marked increase in the activity of the egg, causing a phase of acceleration. This effect was first obtained after eggs had been exposed ten minutes, when it was noticed that divisions had actually been completed in cells where only a spindle was to be seen at the time the exposure began. That is, during an exposure of ten minutes there had been accomplished a complete process which never under normal conditions had been observed in this form to occur in much less than an hour. I have repeated this observation from January to June on many experiments and have obtained the result without variation. Whenever an egg of *Planorbis* in any cleavage up to the sixth, farther than which it is not practical to carry on observations on the living egg, is exposed to X-rays any mitosis which may have been started is hastened to its completion, and in almost every case that state has been reached by the time the egg can be taken from under the tube and examined under the microscope.

Subsequently, I have reduced the length of the stimulation to six minutes, five minutes, and three minutes without noticeable difference in the result. In each case the mitosis (both nuclear and cytoplasmic divisions) in question was nearly if not quite completed at the end of the exposure. Even shorter exposure than this will bring about the result more or less completely. I have exposed the eggs as short a time as twenty seconds and have found the phase of acceleration almost as marked although the cell division would not be fully completed at the end of the exposure. Thus it is seen that a very short exposure only is necessary to induce the acceleration.[1] Comparing this induced result with the normal, it appears that only a small fraction of the time usually taken is consumed under the conditions of the experiment.

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[1] Compare experiment No. 16. (17) At the time of exposure the eggs of this experiment were in the early stages of the second cleavage division, but were not at all in the same stage of advancement. (Some had not quite completed the first division.) The exposure lasted 20 seconds, and at the end of it the eggs were examined as quickly as possible. Many had almost completed the second cleavage and others had passed well into it. Only a few, which were probably in the resting condition at the time of the exposure, showed no effects. Fifteen minutes later nearly all had completed the second division and their nuclei were resting. Forty five minutes later the beginning of the first micromere divisions were visible but

It has been difficult so far to get much information concerning the phase of acceleration. It is passed very quickly; in the last cases cited scarcely more than a minute was consumed, and in no instance was more than ten minutes of exposure necessary to bring about the result. It seems that there is a minimum time in which a cell division in the early cleavages of the egg of *Planorbis* can take place—actually something more than a minute—and that the stimulation by exposure to the rays need be but very short in order to reduce the time from normal to this minimum.

Aside from their bearing on the effect of X-rays on the living organism, the facts connected with the shortening of the time necessary for mitosis have considerable interest from their bearing on the questions of cell mechanism.

The first effect, then, of exposure on the rate of cleavage is to stimulate greatly whatever mitosis may be in progress and to hurry the cells into the resting stage.

The effect of exposing a cluster of eggs not all equally advanced forms an interesting corollary to the observations on the induced acceleration of individual eggs. The usual conditions in *Planorbis* as in most forms, is that all the eggs in a cluster are in the same stages of development. However, there sometimes occurs a variation from the general condition and of the two dozen eggs

thirty minutes yet were consumed before the divisions were completed. An hour and a half later the eggs were in the 12-cell stage.

(18) Eggs in the four cell stage were exposed twenty seconds. Five minutes elapsed before they were examined but at the end of that time the first micromere quartette had been nearly, if not completely, divided off. An hour later the second quartette had appeared and in another thirty minutes the first quartette had divided. Seven minutes later a second exposure of twenty seconds was made, at the end of which in at least part of the eggs the second quartette had divided. It was difficult to see cell boundaries after this stage, but the third quartette could not clearly be seen until after another hour and a quarter had elapsed.

(19) The eggs of the cluster were well along in the second division at the time of exposure, which lasted twenty seconds. When they were examined (as soon as possible after the exposure) the division seemed to be entirely finished. Thirty five minutes later the first quartette was given off, and was followed by the second after another thirty five minutes. A second exposure of twenty seconds was now given; at the end of it, in part of the cells at least, the first quartette had divided. An hour and a quarter elapsed before the end of the next division.

present some one may often be found in nearly every stage of the cleavage division in progress at that time. Reference is here made particularly to the first or second cleavage. But it is very unusual to find eggs in the cluster in two clearly distinct cleavages at once. If a cluster with various stages represented is exposed to the rays, the effect as observed at the end of the exposure is to equalize the progress by hastening all of the eggs, except any which might not have begun the division at all, to the completion of the mitosis and into the resting stage.[1] That is, if the exposure found some of the eggs of a cluster in an early stage of mitosis and others in a later, it would, by inducing the acceleration in each individual bring practically all into the resting stage at the close of the radiation.

It cannot be affirmed, however, from my observations that subsequent divisions of such a cluster as that just described would of necessity occur exactly at the same time.

This observation on a living egg that the divisions are greatly stimulated by the X-rays goes to explain the observed fact that in fixed eggs which have been exposed, mitoses are not so easy to find as in eggs which have not been exposed.

The phase of acceleration does not last long in these cells but passes off at the end of the first division or perhaps the second after the exposure. Following it there sets in without further exposure a phase of depression, during which the rate of cell division becomes slower and slower.[2] The eggs' activity as regards cell division is markedly inhibited. This invariably occurs, although the extent and nature of the inhibition or depression may not be exactly the same in all cases. This depression may amount to a complete stopping of cell division, thus terminating the experiment; or often observation has been interrupted that the eggs might be fixed for cytological examination.

The depression phase occurs without regard to the stage of the development of the egg at which the exposure took place and, so far as now determined, without regard to which particular cleavage is in progress. The whole question of the effect of the rays so far as the rate of division is concerned is not one of the state of progress of the egg in cleavage but one of the mitotic cycle. As long as the exposure is made at the same relative time in the mitosis it makes no difference with which of the early cleavage divisions the experiment begins. It is as though the energy of the eggs was used up upon exposure to the rays in hurrying the eggs into the resting condition and that continuously more and more time is required to raise the cell to the point where it can again divide. Or perhaps it may be plausible to explain the result on the grounds that the X-rays have a double effect: first a stimulative effect, and second, a very injurious inhibitive effect. The former effect is produced during what may be called the latent period of the latter. That there is some particular factor concerned more than mere stimulation is seen from the experiment described below in which a second phase of acceleration and the second phase of depression was obtained by a second exposure. The second stimulation was less and the depression more rapid than the first. Now, if the stimulation were all that had taken place, as for instance in a muscle-lever experiment where the results of *simple* stimulation are obtained, the second stimulus should have been quite as effective as the first; such however was not the case.

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[1] Compare experiment No. 14. (20) The eggs of the cluster were going through the first cleavage division but had not all progressed equally in the division. The exposure lasted twenty seconds, and the eggs had nearly all completed the division at the end of it. Forty minutes later the blastomeres had flattened against each other and an early spindle was to be seen.

[2] Compare experiments (2), (7), and (15).

It has already been stated that very little effect results from exposing the eggs in the resting stage; however, there is some evidence for thinking that such an exposure causes a depression in the rate of division after it. The data at hand on this point are not as positive as desirable, but it seems to indicate that conclusion. In two or three hours after the exposure the divisions became slower and slower as they would have done (but to a much less degree) if the egg had been exposed during mitosis.

There is one case forming an apparent exception to the general observations as stated above. If the exposure occurs during the first maturation division, the depression does not set in until the first cleavage mitosis.[1] The first and second maturation divisions take place with considerable rapidity and show the effects of the stimulation. This, however, is the condition one would expect in view of the fact that a complete mitotic cycle has not elapsed since the exposure, for there is of course no resting vescicular nucleus stage between the maturation divisions. It is, therefore, not a real exception, but on the contrary is quite in line with the other observations.

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[1] Compare experiment (9).

Eggs which have been exposed to X-rays and have passed into the depression phase may again be stimulated by a new exposure to the rays.[1] These exposures may both be as short as twenty seconds and they are both subject to the conditions previously described, but there is no question as to whether the effect will be produced. However, the new phase of acceleration is not so great nor so clearly marked as the first, while the phase of depression comes on sooner and takes place more rapidly than in the case of the first exposure.

The relation which the phase of depression bears to the normal development may be illustrated graphically. The following data, plotted in Fig. 2, are from a representative experiment.

	Control.	Experiment.	Experiment is Faster than Control.
1st div.	75 min.	3 min.	+72 min.
2d div.	55 min.	32 min.	+23 min.
3d div.	80 min.	60 min.	+20 min.
4th div.	70 min.	90 min.	Slower −20 min.
5th div.	65 min.	100 min.	Slower −35 min.

The control or normal is used as a base line and the variations of the exposed eggs from the control are plotted with respect to it. The curve, of course, does not show the phase of acceleration. It indicates what has already been set forth, that owing to the depression the time required for a division gradually lengthens relatively until it is equal to the normal rate, and then falls below. The divisions get relatively slower and slower.

The phase of depression is of sufficient interest to warrant more extended study. To what extent it occurs and how far it may be carried with recovery of the eggs are questions to which I cannot now give a satisfactory answer.

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[1] Compare experiments (18) and (19).

A control,[1] whether part of the same cluster or another cluster in the same stage of development, may be started at the time the exposure is made. If the control is observed after an interval of several hours, it is found ahead of the radiated eggs in development. If, however, the control as well as the radiated eggs be observed at more frequent intervals, quite a different state of affairs is to be seen. During the first two or three divisions after the exposure the radiated eggs are ahead of the control. They gradually get slower, however, as already explained, while control maintains its normal course. Not only do the radiated eggs slow down to the normal rate so that for a very short time both they and the control progress together, but presently they become even slower. Usually by the time the twenty-four cell stage is reached, if the exposure was during the first or second cleavage, the control has more than passed the radiated eggs in the degree of its development. This is explainable on the basis of the observations previously noted. First the divisions of the eggs are stimulated by the exposure, during which time they get ahead of the normal eggs; then the depression phase sets in and

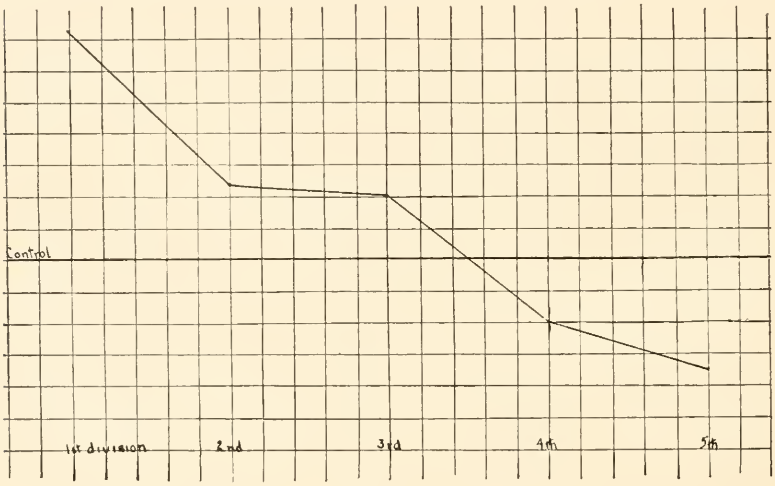

FIG. 2.

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[1] Compare experiments (1) and (7). References to experiments in point might be multiplied greatly, but those given are thought to be sufficient to illustrate the principles set forth.

they gradually get slower and slower until they are developing less rapidly than the normal eggs. Thus the last result is a retardation of growth and cell division.

The following experiment on the eggs of *Physa* gives additional evidence in support of the conclusions just stated and also suggests certain other effects of the rays. There are three sets of data given, and the eggs used were all from the same cluster. All were in the same stage at the beginning of the experiment. In the first column is shown the rate of development of the control; in the second, are the stages for corresponding times of the eggs which were exposed five minutes to the X-rays; and in the third are similar observations for eggs exposed ten minutes. It will be seen that in the shorter exposure the divisions are going more rapidly than the control, but at the end of the experiment they were getting slower. These radiations were made with an apparatus carrying a stronger current and giving off more intense rays than hithertofore. To that condition is without doubt due the effects produced by the stronger radiation. In this last case

Time Intervals.	Control Eggs.	Eggs Exposed to X-rays 5 Min.	Eggs Exposed to X-rays 10 Min.
Beginning of experiment.	All eggs in the early stages of the first cleavage.		
		At the end of the exposure the first cleavage furrow was cutting in.	
+6 min.	1st cleavage furrow not yet visible.	1st cleavage completed.	At end of exposure there was no external sign of division.
+18 min.	1st cleavage furrow beginning to divide the eggs.	Blastomeres flattened against each other.	One egg divided, others show no external signs.
+20 min.	1st division completed.	No record.	Furrow beginning to cut in.
+15 min.	Blastomeres flattening against each other.	4-cell stage nearly completed.	2-cell stage nearly completed.
+130 min.	8-cell stage.	12-cell stage.	4-cell stage.
+4½ hrs.	16–20 cell stage.	24-cell stage.	12-cell stage.

the depression set in before the acceleration could take place and during the entire course of the experiment these eggs were behind both the shorter radiation and the control in development.

As is well known temperature profoundly affects the rate of cleavage, a rise causing an increase in the rate. The question at once is suggested, can the temperature changes induced by the conditions of the experiment account for the effects on the rate as here given. There are several considerations which would seem to point to an affirmative answer to this question. (1) The resistance which the rays meet in passing through the protoplasm of the egg would tend to cause a rise in temperature. (2) It is easily conceivable that some of the energy might be converted into heat energy. Rutherford has shown that radium emanination produces a rise in temperature of considerable extent in gases through which it passes. (This is due, however, to the α rays to the extent of 70 per cent. of the effect noticed). (3) Finally, the histological conditions in the eggs resemble those produced by allowing eggs of *Crepidula* to develop at a temperature six degrees higher than normal (see Conklin, '12). However, in regard to the last point, it is to be remembered that in modifying the development of eggs a given result can often be produced by several different means.

There are on the other hand considerations which make it impossible to account for the effects on the basis of temperature changes. *Planorbis* lays its eggs in shallow water from late winter up into the early summer, and the eggs are adapted to undergo wide changes in temperature without ill effects. They are very small and when the exposures were made were well covered with water in addition to the insulation afforded them by their gelatine and albumen coverings, and furthermore the exposures were of short duration. It is difficult to see, therefore, how sufficient rise in temperature to bring about the marked results herein described could be produced.

Finally, and this seems to be the test experiment, it cannot be that heating causes the effect, because the X-rays produce the acceleration whether the exposure is longer or shorter. There seems to be no marked difference in the effects of a ten minute exposure and one of three minutes, while one of but twenty seconds is almost as effective. Obviously, if rise in temperature were the cause of the more rapid division, a long exposure would give a more rapid cleavage than a very brief radiation.

Recently the writer has been carrying on some experiments, the results of which will be published in another place, to ascertain the effect of X-rays upon certain enzymes. The general conclusion drawn from these experiments is that the activity of the enzymes in question is increased somewhat by a weak exposure, but decreased by a stronger radiation. In the light of the study on cell division here reported and especially of such observations as those of the effects of the stronger radiation on *Physa* eggs, the suggestion of a possible relation between these two sets of phenomena forces itself upon one. In a late paper, Packard suggests "that radium radiations act indirectly on the chromatin and the protoplasm by activating enzymes." This is not unlikely the case, in the writer's opinion, and is in harmony with the observations here presented, for the effects of radium rays appear to be comparable only to those of weak X-rays. It is of course by no means clear how the activation of the enzymes takes place.

SUMMARY OF THE OBSERVATIONS ON EXPERIMENTS.

1. The eggs of *Planorbis* require normally from fifty-five minutes to about two hours to complete a division (up to the twenty-four cell stage). In no case has a division been observed to occur in less than forty-five minutes.

2. By exposure to X-rays during the resting stage of the nucleus only a very slight stimulation may be produced.

3. Exposure during the early part of the formation of the mitotic spindle is most effective.

4. The first effect of exposure upon the rate of cleavage is to stimulate mitotic activity, to bring on a period of hyperactivity. Usually at the end of the exposure the division has been completed, and the cells hurried into the resting stage.

5. Only a very short stimulation is necessary to produce this acceleration.

6. Following the phase of acceleration a phase of depression sets in; the end result is to retard greatly the development of the egg.

7. The depression never follows until a complete mitotic cycle has been passed. Thus if the egg is exposed in the first maturation mitosis the depression does not occur until the first cleavage.

8. An egg may be stimulated during the depression phase by a second exposure to the rays, but the new phase of acceleration is less and the depression follows more rapidly.

9. The control, started at the time of exposure, goes more slowly than the experiment during the first two mitoses, but by the time that the twenty-four cell stage is reached the exposed eggs are progressing more slowly that it.

10. It has not been found possible to account for these effects on the basis of rise in temperature, and the nature of the experiment practically eliminates other disturbing factors; therefore, the effects must be regarded as the result of exposure to the X-rays.

11. Analogy suggests that the effect of X-rays on cell divisions may perhaps be due, partly at least, to the effect of the rays on enzymes contained within the cell.

Discussion of the Literature.

Many observations have been made as to the effect of X-rays and radium on growth and rather diverse results have been obtained. Extensive bibliographies in which the previous work has been quite thoroughly reviewed are to be found in the following publications: Warthin, A. S. (*International Clinics*, 1906, 15th series, Vol. IV.); Gager, C. S. (*Memoirs of New York Botanical Garden*, IV., 1908); Bardeen, C. R. (*Jour. of Exp. Zool.*, Vol. IV., 1908 and *Amer. Jour. Anat.*, Vol. XI., 1911). As far as I have been able to discover, few previous observations have been made on the effect of radiation upon the rate of division. Certain other observations have a less direct bearing but are in line with the conclusions here reached. In view of these facts, it seems necessary to discuss only a few of the papers on this subject.

Bacteria and yeasts have generally been found to be inhibited by exposure to radiation if sufficient stimulus to effect them was given. Koernicke, however, states that if the organisms be transferred to fresh unexposed gelatin, they will grow again, and Gager found budding in yeasts to be increased by exposure.

Certain others of Gager's observations on the effect of radium on plants are concerned with the problem of growth. He reports among his results a cessation of cell division, an acceleration of differentiation, a decrease in cell size, and a lack of coördination in histogenesis. Those processes which go to produce senescence are accelerated.

Guillemont compared the action of X-rays and of the beta rays of radium upon plant cells. He obtained a standard for comparison and found that, the flourescent effect of the two being equal, the beta rays were more intense. The characteristic action is a retardation of the growth, when the rays are fairly strong. He determined also the fatal strength and a comparatively weak strength at which the rays perhaps accelerate.

Becquerel likewise found that weak, or short, stimuli had small effect, while longer ones retarded growth. Exposing seeds for a day had little effect upon their power to germinate, but exposure for a week or more inhibited germination. Pollen germination is also inhibited, according to Lapriore, by exposure to X-rays.

Maldiney and Thouvenin, however, early reported that germination of seeds was hastened by exposure to X-rays.

Gager obtained retardation of growth following exposure of seeds under various conditions. The amount of retardation varied directly with the strength of the radiation. Some kinds of seeds, exposed to radium of weak activity, later showed apparent recovery. It has been shown that hydrogen and hydroxyl ions stimulate germination. Gager says with regard to this, "If the radium rays produce ionization in the mineral solutions in the soil then these ions would act as a stimulus to plants growing there, and, under suitable conditions, cause an acceleration of growth. It is not improbable that the results recorded above are due to a combination of both causes, that is, to the direct action of the gamma rays combined with that of ions produced by the rays in the soil-solution."

Gager in his memoir discussed at length the work done previous to 1908 upon both plants and animals. The results upon which there is any very general agreement, he summarizes in the following eight statements:

"1. Radium rays have the power to modify the life-processes of both plants and animals.

"2. Röntgen rays and radium rays produce similar physiological results.

"3. Sensitiveness to these rays varies with the species of either plant or animal.

"4. Younger, and especially embryonic tissues, are more sensitive than those more mature.

"5. With only one or two exceptions, exposure to radium rays has been found to either retard or completely inhibit all cell-activities. The rays may cause irregularities in mitosis.

"6. Experimental evidence for or against the existence of a radiotropic response is conflicting.

"7. Whatever the immediate, internal change produced in the protoplast may be, the result, with animals as well as with plants appears to be more or less profoundly modified by the presence of chlorophyll in the cell.

"8. Radium rays appear to retard the activity of enzymes."

Gager suggests in his final discussion of the results in his memoir (p. 271) that "the rays may operate so as to increase or decrease the amount of energy available for the work" (meaning metabolic processes) "and, lastly, variations in growth may be, either wholly or partly, expressions of the influence of the rays on cell division." In the latter case growth would be an index as to the effect of the rays on the reproductive functions of the cell, and this, it would seem, is highly probable.

He says also, "No one has yet succeeded in accelerating the rate of cell division or in increasing its amount in a given tissue by means of radium rays. The only results recorded are the introduction of irregularities and complete inhibition." After some discussion of this he says further, "Thus we should expect *a priori*, a retardation and finally a complete inhibition of cell division in all tissues exposed to rays of sufficient activity and for a suitable period of time. And this is what has been observed to occur. Theoretically we ought also to be able to accelerate the process by suitable conditions of exposure, but such conditions have not yet been discovered." Whatever the effect of radium, that of X-rays is most positive in regard to its ability to accelerate division, at least under certain conditions.

His final paragraph is also of interest in this connection. He says:

"The broadest, and at the same time the most definite generalization warranted by the work so far done is that the rays of radium act as a stimulus to metabolism. If this stimulus ranges between minimum and optimum points, all metabolic activities, whether constructive or destructive, are accelerated; but if the stimulus increases from the optimum toward the maximum point it becomes an over-stimulus, and all metabolic activities are depressed and finally completely inhibited. Beyond a certain point of over-stimulus recovery is impossible, and death results."

Another statement of the same fact is given on page 157: "The rays of radium act as a stimulus to protoplasm. Retardation of growth following exposure to the rays is an expression of over-stimulation, acceleration of growth indicates stimulation between a minimum and an optimum point."

Protozoa present some variation in their reaction to X-rays. Some are killed but others are very resistant and appear little disturbed. Some are much more susceptible than others. In some cases cytoplasmic and nuclear activities are affected, while in other cases such a process as conjugation goes on apparently unaffected. There is no positive evidence of tropic responses to radiations. Bardeen reports that *Paramœcium* may be exposed as much as twelve hours without disturbing conjugation, or the rate or forms of division.

The effect of radium upon *Ascaris* eggs has been studied by Perthes, by Barlow and Bonney, and by Paula Hertwig. The former found that after exposure the cell divisions became slower and more irregular than the control, and finally gave rise to irregular cell masses or misshapen little worms especially abnormal at the posterior end. The controls, however, gave rise uniformly to active worms. The eggs in the resting and dividing conditions were equally affected, and the degree of exposure is the factor upon which the result depends. Nuclei and in particular the chromatic structures were most injured, while spindle and centrosomes appeared quite normal. The chief effects did not appear at once upon stimulation but only after a certain period of time had elapsed. The results obtained from X-rays were entirely analogous to those from radium.

Barlow and Bonney, studying "the influence of radio-activity on the division of animal cells," found in *Ascaris* eggs a retardation of the early cell divisions which was followed by death. According to them, a short radiation causes an acceleration of division.

Fräulein Hertwig investigated the effect of radium on *Ascaris* eggs to find evidence as to whether the chromatin and other nuclear structures are directly affected by radiation, as claimed by O. and G. Hertwig, or whether the rays act to break down lecithin and affect the chromatin only indirectly, as held by Schwarz, Schaper and others. Her evidence goes to support the former view. Furthermore, she is not in agreement with all previous work on this form. She finds cytological evidence that even in the first division after exposure the chromatin is affected, although Perthes speaks of what might be regarded as a latent period. She agrees with Barlow and Bonney that exposure causes a retardation, but was unable to secure acceleration of the divisions even with so short an exposure as five minutes. The amount of retardation depends to some extent on the length of radiation, eggs radiated one hour with a given preparation developing farther than those radiated two hours.

Negative results in exposing eggs of sea urchins to X-rays have been reported by both Schwarz and Bardeen, but the recent brilliant studies of Gunther Hertwig as well as the older paper by Bohn on the effect of radium on these forms make it desirable to repeat the experiments with X-rays. Hertwig's results, like those of Fräulein Hertwig, were chiefly concerned with the behavior of nuclei of exposed eggs and his results are convincing along that line. During the course of his experiments he noted that the progress of division in the sea urchin eggs, which had been fertilized with sperm exposed to radium bromide rays, was very greatly retarded even from the first cleavage. At the end of the second day most of these eggs had died, after a decidedly irregular course of development. His most important results do not bear upon the question here under discussion.

Bohn found that an exposure to radium of forty minutes accelerated segmentation in eggs of the sea urchin, although a longer exposure retarded it.

The only previous experiments upon Gasteropods, so far as I am aware, are those of Tur upon the development of eggs of the snail *Philine* after exposure to radium. He states that in eggs exposed before the first division the segmentation was in no wise delayed by the action of the radium, but that the cleavage as well as gastrula formation was normal. Only later did the effects of radium show themselves. My results do not confirm these observations.

Congdon studied the effect of the beta rays upon several forms. He found that an exposure of twenty-four hours caused a retardation of 31.2 per cent. in the development of the eggs of *Drosophila*. The more intense the radiation the greater the retardation. In these experiments the intensity was measured by the distance of the object from the radium. "Secondary beta radiations (slow electrons) produce a much stronger effect than primary radiations (rapid electrons) of like intensity." He experimented upon Tubularia varying the length of the exposures. Both in *Drosophila* and in the hydranths, he states, "many stimuli which retard or stop growth if of high intensity will accelerate it if they are weak enough." The retardation varies directly as the length of the exposure. "When the fundaments of regenerating *Tubularia* hydranths were exposed to beta radiations from three hundred milligrams of impure radium one thousandth as strong as the pure bromide for periods up to three days in length, the shorter exposures were found to accelerate regeneration and the longer to retard. The degree of retardation increases slowly with lengthening exposure; but the degree of retardation relative to the length of exposure decreased with lengthening exposure."

Again he found that seeds were most sensitive to radiation when the embryos were turned toward the radium. Here also the slower electrons of the beta radiations were more effective relatively than the more rapid.

Zuelzer also reports that insects are affected by exposure to radium.

The vertebrates have served as objects for a large part of the experiments with radium and X-rays. Gilman and Baetjer exposed hen's eggs for ten minutes daily to X-rays. During the first thirty-six hours the development was accelerated. Then there followed a retardation during which the development was greatly altered as well as checked.

Comparable results were obtained by these same investigators working on the eggs of *Amblystoma.* Exposures of fifteen minutes daily first produced a period of acceleration which lasted up to ten days in some embryos, but at the end of four days abnormalities began to manifest themselves. Up to the tenth or eleventh day the exposed eggs were larger than the controls; after that they grew no larger, some became actually smaller, and all were grotesque. The controls on the other hand continually grew larger.

In other eggs which were exposed daily four or five times, but otherwise permitted to develop undisturbed, the tendency to recover and develop normally was noted. This was not a clear result, however, for in less than half of the eggs so exposed was restitution of form affected, and all died after the exposure of the twenty-third day.

The occurrence of a latent period is reported by Schaper as one of the results of exposing eggs of *Rana fusca* and of *Triton* to radium. During the first day of his experiments no departure from the normal course of development was noticed. Following this "latent period," Schaper observed that the development of the embryos was greatly interfered with, marked abnormalities and finally death being produced. The duration of the latent period depends upon the intensity of the radiation and upon the state of development of the embryo at the time of exposure. In nearly all cases it lasted a day, and if older larvæ were used, with relatively short radiation, it might last several days. The course of development was always more or less drawn out, passing into a condition of standstill to be followed at last by death. In general, Schaper found that there were inhibitive effects on cell division, embryonic differentiation, and embryonic growth.

Bardeen has found by exposing either sperm, or eggs before fertilization, or fertilized eggs to radium that abnormalities are produced and he proceeds upon the hypothesis that the nuclei are affected, thus causing the retardation in growth. "Cleavage in most eggs fertilized by exposed sperms seemed to be normal. In several of the experiments it appeared to be slightly more rapid than in the control eggs." In mature eggs which were exposed to X-rays and then fertilized with normal sperm "the early cleavage stage appeared to be normal."

He also says, "It would appear as if the nuclei in mitosis were forced into a resting stage in the spindle by the X-rays."

Finally, O. and G. Hertwig have carried on a thoroughgoing series of studies on the influence of radium upon developing eggs and larvae of amphibians. This set of observations includes first, the nature of the pathological changes due to radiation which are found to occur in the various organ systems and in the body form, and second, the effects of the radiation upon the nuclei and cytoplasm of the various tissues. The evidence is found to support the view that the nuclei are injured directly. In the early stages injury and general retardation of development take place; the effect, however, on the rate of cell division is not discussed, I believe, by either writer.

LITERATURE.

Bardeen, C. R.

'09 Variations in the Susceptibility of Amphibian Ova to X-Rays at Different Stages of Development. Anat. Record, Vol. III.

'11 Further Studies on the Variation in Susceptibility of Amphibian Ova to X-Rays at Different Stages of Development. Amer. Jour. Anat., Vol. XI.

Barlow, Lazarus, W. S. and Bonney, Victor.

'09 The Influence of Radioactivity on the Division of Animal Cells. Archives of Middlesex Hospital, Vol. 15. (Accessible to me only through reviews.)

Becquerel, H.

'01 Sur quelques effets chimiques produits par le rayonnement du radium. C. R. de l'Acad. de Sci., T. 133, p. 709.

Bohn, G.

'03 Influence des rayons du radium sur les oeufs vierges et fécondés, et sur les premiers stades du dévellopment. C. R. de l'Acad. de Sci., T. 136, p. 1085.

Congdon, E. D.

'12 The Influence of Radiations of Radium upon the Embryonic Growth of the Pomace-fly, Drosophila ampelophila, and upon the Regeneration of the Hydroid, Tubularia crocea. Bull. Mus. Comp. Zool., Vol. 53, No. 7.

'12a. A Comparison of the Alterations in the Velocity of Growth of Certain Seedlings through the Action of Rapid and Slow Electrons of the Beta Rays of Radium. Arch. f. Entw-m., Bd. 34.

Conklin, E. G.

'97 Embryology of Crepidula. Jour. of Morph., Vol. 13.

'02 Karyokinesis and Cytokinesis. Jour. Phila. Acad. Nat. Sci., Vol. XII.

'12 Experimental Studies on Nuclear and Cell Division in the Eggs of Crepidula. Jour. Phila. Acad. Nat. Sci., Vol. XV.

Gager, C. Stuart.

'08 Effects of the Rays of Radium on Plants. Mem. N. Y. Bot. Garden, IV.

Gilman, P. K. and Baetjer, F. H.

'04 Some Effects of Roentgen Rays on the Development of Embryos. Amer. Jour. Physiol., X., p. 222.

Guilleminot, H.

'07 Effects comparés des rayons X et des radium sur la cellule végétale. C. R. de l'Acad. de Sci., T. 145, p. 798.

Hertwig, Gunther.

'09 Radiumbestrahlung unbefruchteter Froscheier und ihre Entwicklung nach Befruchtung mit normalen Samen. Arch. f. Mikr. Anat., Bd. LXXVII., Abt. 2.

'11 Das Schicksal des mit Radium bestrahlen Spermachromatins in Seeigelei. Arch. f. Mikros. Anat., Bd. LXXIX., Abt. 2.

Hertwig, Oskar

'09 Die Radiumkrankheiten tierischer Keimzellen. Arch. f. Mikros. Anat., Bd. LXXVII., Abt. 2.

Hertwig, Paula

'09 Durch Radiumbestrahlung hervorgerufene Veränderungen in den Kernteilungsfiguren der Eier von Ascaris megalocephala. Arch. f. Mikros. Anat., Bd. LXXVII., Abt. 2.

Holmes, S. J.

'00 The Early Development of Planorbis. Jour. Morph., Vol. 16.

Koernicke, M.

'04 Ueber die Wirkung von Röntgenstrahlen auf die Keimung und das Wachstum. Berichte der Deutschen bot. Gesellsch., Bd. XXII.

'05 Weitere Untersuchungen über die Wirkung von Röntgen und Radiumstrahlen auf die Pflanzen. Ibid., Bd. XXIII.

Levy, O.

'06 Mikroskopische Untersuchung zur Experimenten über den Einfluss der Radiumstrahlen auf embryonale und regenerative Entwicklung. Arch. f. Entw-m., Bd. XXI.

Maldiney and Thouvenin

'98 De l'influence rayons X sur la germination. Revue gen. de Bot., T. X.

Packard, Charles

'14 The effect of Radium Radiations on the Fertilization of Nereis. Jour. of Exp. Zoöl., Vol. XVI.

Perthes, G.

'04 Versuche über den Einfluss der Röntgenstrahlen und Radiumstrahlen auf die Zellteilung. Deutsch. med. Wochensch., Nr. 17 und 18, Bd. 30.

Schaper, A.

'04 Experimentalle Untersuchungen über den Einfluss der Radiumstrahlen und der Radiumemanation auf embryonale und regenerative Entwicklungsvorgänge. Anat. Anz., Bd. XXV.

Schaudinn, F.

'99 Über den Einfluss der Röntgenstrahlen auf Protozoen. Arch. f. Ges. Physiologie, Bd. LXXVII.

Schwarz, G.

'03 Uber die Wirkung den Radiumstrahlen. Archiv f. Physiol., Bd. 100.

Tur, J.

'09 Sur le développement des oeufs de Philine aperta L. exposés l'action du radium. C. R. de l'Acad. des. Sci., T. 149.

Zuelzer, M.

'05 Ueber die Einwirkung der Radiumstrahlen auf Protozoen. Archiv. f. Protist., Bd. V.

HABITS OF THE LARVA OF BELLURA MELANOPYGA GROTE (LEPIDOPTERA).[1]

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Of the great host of *Lepidoptera*, only a few species have invaded the water and acquired aquatic stages in the life history. These few species have solved the problems of maintenance of aquatic life in exceedingly interesting ways, all presenting unique adaptations. The species on which this paper is based is aquatic in the larval stage and rivals the other species of similar habit in the character of its peculiar adaptations.

In 1881 Comstock ('81, pp. 147–149) published a short paper which included Grote's description of a new species, *Arzama melanopyga* (now *Bellura melanopyga*), and a very brief account of the larva of the same, presenting data on its unique habits and adaptations. This seems to be the only published account, the writer having searched in vain for other papers dealing with the habits of this larva. Comstock later ('88, p. 468) made mention of it and Grote ('89, p. 226) called attention to its habits and structures but in neither case were new data presented. The larval habits of a closely allied form, *Sphida obliqua* Wlk., which feeds on *Typha*, are better known and in a few respects resemble those of *Bellura melanopyga*. The following paper includes new data on the unusual habits of the larva and presents the detail of features merely mentioned by Comstock. *Bellura melanopyga* Grt. has been considered by some writers as a synonym of *Bellura gortynoides* Wlk. but the writer follows Hampson ('10, p. 260), who considers *melanopyga* a valid species.

The observations which form the basis of this paper were made by the writer while a member of the staff of instruction at the University of Michigan Biological Station at Douglas Lake, Michigan, during the three sessions of 1911–1913. The abundance of material and the opportunity to make observations at corresponding times during successive seasons made it possible to verify the data and to carry on some phases of the work which otherwise would have been impossible. The observations extended over the months of July and August of each of the three years. Larvæ of *Bellura melanopyga* began to appear during the first week of July and could be found until late in August. They feed on the leaves and burrow lengthwise into the petioles of the yellow waterlily, *Nymphæa americana* (Provancher) Miller and Standley ('12, p. 78). This lily occurs in considerable abundance in the beach pools and the protected bays of Douglas Lake. It also occurs in almost every sphagnum bog in the surrounding territory. This distribution seems to be dependent upon the fact that only at these places are attained the chief conditions which favor their growth, namely, protection from winds and waves, shallow water, and a more or less mucky bottom. *N. americana* was very abundant in one of the inlets (Bessey Creek) and the larvæ of *Bellura melanopyga* were correspondingly numerous, the infestation in 1912 being 90–95 per cent. The percentage of infestation in the beach pools and in the protected bays was much lower, not exceeding 25 per cent. This was probably due to the fact that Bessey Creek is much better protected from wind and waves, factors which are unfavorable to the very young larvæ. The lilies growing in the bogs of the surrounding region showed no signs of infestation.

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[1] Contributions from the University of Michigan Biological Station No. 22.

Feeding Habits.

The feeding habits of this larva were studied in considerable detail. These activities vary considerably according to the age of the larva. Two fairly well-defined periods can be recognized (1) a very early period, the *leaf-feeding period*, or the *mining period*, which lasts approximately throughout the first two stadia, and (2) the later period, the *petiole period*, which includes the remainder of the larval existence.

The Leaf-Feeding or Mining Period.—The eggs have not been observed but it is evident that they must of necessity be laid somewhere on the leaf since, as will be shown in a later discussion, the very young larvæ are not efficient swimmers and were often found in places where it would have been impossible for them to get had the eggs been laid elsewhere. Furthermore, at the beginning of the season it was no uncommon thing to find twenty or more very young larvæ, 3–4 mm. in length, on a single leaf. The presence of so many very young larvæ on one leaf and the absence of larvæ from a contiguous leaf is taken as evidence that the eggs were laid somewhere about the infested leaf.

During the mining period the larva works on the upper side of the leaf. It usually cuts a somewhat circular hole, slightly larger than itself, through the upper epidermis and penetrates into the parenchyma, thus becoming a *miner*. There is no regularity in the shape of the mine. Sometimes it appears as a winding tunnel with a diameter about twice that of the larva; sometimes it is digitate in appearance; and sometimes it resembles a "blotch mine." The area included in each may vary to considerable extent. Holes through the epidermis other than the original entrance may occur anywhere throughout the length of the mine. Mines are easily detected on the surface of a leaf since they soon become whitish in appearance, due to the removal of the chlorophyll-bearing tissue. They may occur anywhere on the leaf. Leaves were sometimes found in which the mines appeared to extend either towards the midrib or towards the junction of the midrib with the petiole but an examination of a large number of infested leaves leads the writer to believe that no importance can be attached to these cases.

The initial entrance to a mine is usually surrounded by excrement and a small quantity of finely masticated leaf tissue. Microscopical examination shows that the latter is composed of the broken fragments of the epidermal cells and numbers of the peculiar *idioblasts* which are so common in the tissues of the yellow waterlily. Examination also shows that the fragments of the epidermal cells have not undergone digestion and furnishes evidence for believing that the larvæ never use the epidermal tissue as food but simply remove it with the mandibles. The excrement is greenish when first voided but gradually becomes lighter in color until finally all color is completely lost. It is voided in the form of small, somewhat oblong, uniform masses which tend to remain together, forming chains of varying lengths. Microscopical examination shows that it is composed of two kinds of elements: (1) idioblasts, which form as much as 60 per cent. of the excrement and show no evidence of being affected by digestion, and (2) greenish, unorganized matter, which represents the remains of the digested parenchymal tissue.

Even in the very early stages the larvæ are active feeders. Larvæ, 5½ mm. long, constructed mines 14 mm. long in less than 20 hours. Larvæ, 7 mm. long, when transferred to new lily leaves completely buried themselves in four hours. Usually but one larva occupies a mine. Several instances were observed, however, in which two were occupying the same mine and in one heavily infested leaf eight larvæ of about the same size were found in a single, large mine. The formation of the mine is due primarily to the fact that the larva apparently uses only the parenchyma as food and must get under the epidermis in order to get it. However the mine has an indirect value since it furnishes a protection for the larva during the early and more helpless stages.

Several days after the mine has been formed the upper and lower epidermis bounding it begin to disintegrate, untimately leaving an ugly hole in the leaf and, in badly infested leaves, producing numerous perforations. The effect on the plant is evident. Not only is the leaf disfigured but in proportion to the number of holes present the leaf surface is also reduced. Many cases were observed in which the infestation was great enough to cause the death of the entire leaf.

In spite of the fact that in the Douglas Lake region a goodly number of other insects affect the yellow waterlily some of which also produce holes and burrows in the leaves, it is not difficult to identify the work of young *Bellura* caterpillars. Certain beetles (*Donacia*) make holes through the leaves in order to deposit eggs but these are distinguished by the circular shape of the holes, and by the rows of eggs or the remains of the eggs on the lower side while the holes made by the larvæ of *Bellura melanopyga* are usually irregular and bear no eggs on the lower margin. The larva of another beetle (*Galerucella nymphæa*) works on the upper side of the yellow waterlily leaf but instead of making mines it produces irregular trenches on the surface by eating away the epidermis, giving the surface a brownish, etched appearance.

The Petiole Period.—This period begins when the larva quits the mining habit and begins to burrow lengthwise down the petiole. It is initiated when the larva locates either the midrib, or the junction of the leaf with the petiole, and from this time the feeding activities are confined almost entirely to the petiole. The latter is usually reached in one of two ways: (1) the larva burrows through the leaf substance or wanders on the surface until it reaches the midrib, whereupon it bores into it, tunneling towards the attachment of the petiole, or (2) the larva burrows or eats through the substance of the leaf or wanders on the surface until it reaches the attachment of the petiole where it then begins to excavate. At about this time the larva has attained such a size that mines can no longer be made but instead a broad slit in the leaf is produced. This slit (Figs. 3–5) is usually a trifle wider than the diameter of the larva producing it and almost invariably extends directly towards the midrib or its junction with the petiole. Sometimes a number of these slits are made before the larva constructs one which reaches into the substance of the midrib but all extend in the same general direction. If the midrib is reached first the larva bores lengthwise into it and towards the junction of the leaf with the petiole. Only very rarely were larvæ found boring in the opposite direction. The work in the midrib is merely preparatory to the work in the petiole, being only a means of getting into the upper end of the latter. It is not possible at present to account for the ability of the larva to recognize the position of the midrib or the petiole from any position on the surface of the leaf, and to distinguish between the apex of the midrib and the attachment of the petiole.

As will be shown later active dissemination of the larvæ begins at the initiation of the petiole period. Many instances were observed in which a larva had come to a new leaf and with surprising exactness had gone directly to the junction of leaf and petiole and had burrowed into it. Other similar instances were observed in which the larva on reaching a new leaf had cut a preliminary slit through the leaf leading directly to the top of the petiole. This ability to work directly from the periphery of a new leaf to the region of the petiole is especially marked in larvæ over 3 cm. in length. These facts were made apparent many times by field experiments in which larvæ 4 cm. in length were removed from their burrows and placed on other leaves; also whenever it was necessary to restock the large tank, which served as the aquarium, by introducing larvæ from the field. In about 40 per cent. of these cases the larvæ bored into the petiole without any preliminary cutting of the leaf while others cut slits leading directly towards the attachment of the petiole. With the beginning of the petiole period comes the restriction of a single larva to each leaf.

Length of the Burrows.—The length of the burrow in the petiole varies according to the time it has been inhabited, and to some extent according to the size of the larva. Burrows were frequently two feet long and occasionally longer. Sometimes the burrow extended down to the rootstalk but no instance was observed in the field in which the burrow extended into it. There seems to be no reason why this should not occur since it will be shown later that the substance of the rootstalk can be used as food.

Deserted Burrows.—Burrows of various lengths were often found which had been constructed and then deserted without apparent cause. These burrows ultimately collapsed to some extent and became brownish on the inside. Usually they were inhabited by other aquatic animals, such as gyrinid larvæ, crustaceans (amphipods), chironomid larvæ, small aquatic coleoptera, and small leeches.

Formation of New Burrows.—In order to observe the initial steps in the production of a new burrow, larvæ 4½ cm. in length were removed from the petioles and placed in the aquarium on fresh, uninfested leaves. Some took to the leaves and began boring from the upper side of the leaf into the petiole while others attacked petioles which happened to be nearly horizontal and only partly under water. The larva began excavation by biting into the tissue until the mouth was full; then the head was withdrawn and the mass expelled at the margin of the hole. This was continued until the head was buried after which time the larva ate the tissue removed. Microscopical examination of the initial material deposited around the margin of the hole showed that it was merely pulverized leaf tissue.

Other Points of Attack.—Occasionally peduncles were found both in the field and in the aquarium which had been attacked.

In all cases the initial point of attack was at the top of the flower. Both opened and unopened flowers were affected. Floating rootstalks in the aquarium were always attacked and, if left for any length of time, were ultimately excavated through and through. This utilization of the substance of the rootstalk was not always caused by famine. A number of specimens were kept under conditions where rootstalk substance alone could be secured as food and apparently thrived on it. It thus appears that such materials may serve as food for these larvæ.

It sometimes happened that a fall in the level of the water caused portions of the long petioles to be bent above the surface of the water, or at least to lie on the surface. Whenever larvæ discovered these emergent petioles they attacked them, making an entrance through the side of the petiole.

Excrement.—In the field the best mark of recognition of the work of these larvæ is the heap of excrement which accumulates around the margin of the burrow on the upper side of the leaf. As would be expected the quantity depends upon the length of occupancy and the activity of the larva. In August it was a common thing to find hundred of leaves with conspicuous heaps of excreta around the hole on the upper surface.

The excrement is always deposited outside of the burrow regardless of the relation of its opening to the level of the water. Since the position of the larva is such that the posterior end is towards the leaf, the excrement is always deposited by thrusting the terminal somites out through the entrance of the burrow. That the level of the water has nothing to do with determining the place of deposition of excrement is shown by the following observations: (1) In floating, partially submerged rootstalks which had been tunneled by the larvæ it often happened that much of the upper part of the burrow contained no water but invariably all of the waste matter was deposited on the outside around the entrance. (2) Instances were observed where the entrance to the burrow was submerged for about an inch, due to a raising of the level of the water. The waste matter was always deposited at the entrance of the burrow and under water.

Microscopical examination of the excrement at all stages of the petiole period showed a composition similar to that described for the leaf-feeding period. The percentage of idioblasts was large and they showed no indication of having been affected by the digestive process. The majority of the idioblasts were intact. Some showed signs of fragmentation, due to the initial action of the mandibles when the plant tissue was removed. Examination of the excrement also showed that, except for a short time when the larva is cutting away the epidermis and surrounding tissue in starting a new burrow, all of the material which is excavated passes through the alimentary canal of the larva and is subjected to the digestive process. Thus it is evident that the length of a burrow is a criterion of the amount of food material which has been taken from the plant. The larva is a voracious feeder, eating during both day and night, and large quantities of plant tissue are consumed.

Effect on the Food Plant.—The effect of the larva is such that infested plants are doomed. During the latter part of July and early August hundreds of leaves showed incisions converging towards the midrib and more especially towards the attachment of the petiole. Many leaves turned yellow and disintegrated, due to one or both of two causes: (1) eating in the region of the attachment of the petiole almost or entirely severed the connection of the leaf with the petiole; (2) the removal of the greater part of the substance of the petiole and the occasional eating through one side produced a more or less complete separation of the leaf from the rootstalk.

It thus appears that these larvæ produce havoc in the beds of *Nymphœa americana* and are a serious enemy since they not only disfigure the leaves but also actually cause the destruction of the same. They also destroy the flowers to some extent. In fact, of the goodly number of insects found on *N. americana* in the Douglas Lake region, the *Bellura* larvæ are usually the worst enemy. Fortunately the rootstalks seem to suffer but little from their attacks.

Other Plants as Food.—Observations and experiments showed that under normal conditions these larvæ use only the yellow waterlily as food. In the field none were found feeding on the white waterlily (*Castalia odorata*) notwithstanding the fact that in many cases *Nymphœa americana* and *Castalia odorata* occurred in the same locality, frequently intermingling and contiguous. In the region studied *Castalia odorata* is surprisingly exempt from insect attacks. Beutenmüller ('02, p. 440) states that, in the vicinity of New York City, the caterpillar of *Bellura melanopyga* "bores in the leaf-stalks of the common white pond lily and yellow pond lily." Assuming that the "common white pond lily" referred to above is *Castalia odorata*, it appears that the larva may work normally on it in certain other localities. However, the evidence collected in the Douglas Lake region is entirely contradictory.

Experiments were carried on at the laboratory with the view of determining whether or not these larvæ could be forced to use other food. The following are typical experiments and results: *Experiment 1.* Two larvæ, each 6 mm. in length, were transferred to a watch glass containing a piece of leaf of the white waterlily (*Castalia odorata*) which was kept moist. During the first eighteen hours the larvæ wandered restlessly about, then finally began working on the white waterlily leaf and at the end of four hours one had completely buried itself and the other was making similar headway. Fresh pieces of the white waterlily leaf were used to replace the eaten ones from time to time and these larvæ were kept thus for twelve days. At the end of that time both were alive, apparently in healthy condition and one ecdysis had occurred. The results show that it is possible for the larvæ to use the white waterlily as food when forced to do so by the absence of the normal food. *Experiment 2.* Both yellow and white waterlilies were placed in the same aquarium which was stocked with several larvæ, 4 cm. in length. The larvæ attacked the yellow waterlilies and worked on them continuously. Ultimately the food plants were consumed and a famine was allowed to occur. After a lapse of several hours only one case was observed where larvæ were attempting to tunnel into the petiole of one of the white waterlilies and it was finally abandoned, the larvæ wandering restlessly about the aquarium. *Experiment 3.* Larvæ were placed in covered dishes containing *Potamogeton natans* and *Sagittaria* sp., plants which occurred in abundance in the same habitat with the waterlilies, and were left thus for several days. The tissues of these plants were invariably and constantly refused.

RESPIRATION.

One of the problems which confront an insect possessing an aquatic stage is that of securing the requisite amount of oxygen. Those few genera of *Lepidoptera* which are unique in having aquatic larvæ have solved the problem in one of two ways: (1) by the utilization of the dissolved oxygen, either by means of gills or through cutaneous respiration, or (2) by making periodic trips to the surface in order to secure the oxygen from the air. Each of these adaptations calls for distinctly differentiated structures and each is accompanied by interesting habits and activities. *Bellura melanopyga* has solved the oxygen problem by a peculiar development of the spiracles and by making *periodic trips to the surface.* A careful study has been made of this adaptation and the results will be given in some detail.

Respiratory Apparatus.—Paired spiracles are present on I, IV, V, VI, VII, VIII, IX, X, and XI. All, exclusive of the pair on XI, are similar in size, are elliptical in shape, are lateral in position, and have the long axis vertical. Somites I–X are similar but XI–XIII form the chief structural differentiation accompanying this aquatic habit. XII is very short, being only about one-fourth as long as XI, and its dorsal surface is depressed considerably below the corresponding surface of XI. The dorsal, posterior margin of XI bears two large, elliptical spiracles, one on each side of the median line. They are placed obliquely, the long axis being inclined about forty-five degrees from the vertical. They are at the posterior termini of the lateral, longitudinal tracheal trunks, opening directly into them. All other spiracles have short branches leading into the longitudinal trunks. Each of the terminal spiracles on XI opens into a somewhat enlarged region of its respective longitudinal trachea which probably serves as a reservoir for the supply of air which is carried below the surface by the larva on its downward trips. XIII is somewhat larger than XII and forms the cauda. Its dorsum is on the same level with that of XII and the lateral margins converge caudad.

The Leaf-Feeding Period.—During the leaf-feeding period respiration is carried on in the same way as in terrestrial lepidopterous larvæ. Each larva is working in a mine in the leaf but the entrance hole and other openings which usually occur are sufficient to provide the necessary air.

The Petiole Period.—When the larva deserts the leaf and becomes a borer in the petiole new conditions are encountered and new provisions must be made. The leaves of *Nymphæa americana* are never normally held above the surface of the water by the petiole as in the case of *N. advena* but the emergent leaves float on the surface. This means that the burrow in the petiole is filled with water and that the larva is submerged. When the length of the burrow increases to such an extent that it is longer than the body of the larva which is making it, the latter is compelled to resort to other means of getting air. To do this the larva makes *periodic trips* to the surface where the fresh air is drawn into the tracheal system in sufficient quantity to allow a sojourn of several minutes under water at the bottom of the burrow.

As stated previously, the larva lies in the petiole with the posterior end towards the top of the burrow. This position eliminates the necessity for the larva to come out of the burrow when taking air, and to turn around when starting on its trip to the top. When at the bottom of the burrow the larva feeds (or in some cases merely rests) until the need for air stimulates it to return to the surface. Then it *backs up* to the top of the burrow, stopping when the large pair of spiracles on the posterior margin of XI is just pushed above the surface film. It remains in that position until sufficient air has been taken into the trachea to permit a return to the bottom of the burrow again. This alternate sequence of feeding and breathing goes on continuously so long as the larva remains in the burrow. In this form of respiration it appears that the other spiracles are not needed since only the posterior pair is pushed above water.

Frequency of Trips to the Surface.—A number of observations were made on various larvæ in order to determine what is the normal period of time spent under the water and likewise the normal period spent at the top. The records were made by means of a stop-watch and the observations on each larva were carried on long enough to secure data showing the range of variation in each case. The average of 128 observations involving 13 different larvæ, 4–6 cm. long, showed that the interval spent below the water was 2 minutes and 57 seconds, and the time interval spent at the top was 1 minute and 21 seconds. The maximum period of time voluntarily spent below was 13 minutes while the minimum was 20 seconds. The maximum period at the top was 6 minutes and 40 seconds, and the minimum was 5 seconds. Comstock ('81, p. 148) states that he observed larvæ of this species remain under water for a half hour but in the large number of observations made in this connection none remained below over 13 minutes.

In connection with the observations on the frequency of trips to the surface, the question arose as to whether there was any correlation between the length of the burrow and the length of time between appearances at the top. In order to determine this point observations were made on a number of larvæ from day to day as they increased the length of their burrows. Results show that the length of the burrow does not in any way determine the time spent below the water. In fact, they show that the intervals are approximately the same when the burrow is but 6 cm. long as when it is over 20 cm. in length. From an examination of all results secured in connection with this study, the writer is convinced that the length of the periods spent under water is purely a matter of supply and demand of oxygen, that it is dependent upon the amount of air which the larva draws into the trachea when at the top and the activity of the larva in the process of feeding at the bottom of the burrow.

Resistance to the Lack of Oxygen.—Experiments were carried on to determine how long larvæ can live under water, when forced to do so, without renewal of the air supply. The entrances to the burrows were plugged in such a way that the inmates could not get air at the top, and the time recorded. These petioles were visited at regular intervals and the condition of the larvæ noted. Larvæ were also placed in tubes of water which were corked so that no air spaces were left at the top, and the condition of the specimens carefully observed. These experiments showed that death usually occurred after an interval of about two hours. After 15 or 20 minutes of continued submergence larvæ began to show the first signs of uneasiness and evidences of weakness began to appear at the end of an hour.

In this connection another set of experiments was carried on in which the infested lily leaves were staked down under water to a depth of six or seven inches and the behavior of the larvæ under these new conditions noted. Each larva came to the top of the burrow and gradually pushed the posterior end out through the entrance, continually searching in a random fashion for the surface film. Ultimately it loosened its hold, came to the surface, and swam about until it came to another lily leaf. In some cases larvæ lived for 45 minutes under water before they would release the hold and come to the top.

Expired Air.—After a larva has been at the bottom of a burrow for a brief interval one or two bubbles usually rise to the top. This happens commonly at the end of the period below and just before the beginning of the trip to the top. These bubbles probably represent, in part at least, expired air which is expelled from the tracheæ.

The Breathing Position.—Except when the larva voluntarily leaves the burrow, it only tips the surface with the posterior end when taking air. Normally only the dorsal surface of XII and XIII and just enough of the posterior margin of XI to include the large dorsal spiracles is above the surface film, the other portions of the body remaining under water. When the larva comes to rest at the breathing position the terminal spiracles pull down the surface film, forming a small conical depression. When in this position the larva is very sensitive to surface disturbances and responds quickly to them by retreating into the burrow. Thus in making observations on the breathing activities it was very necessary to avoid making even the smallest ripples or swells. Very tiny jars on the leaf produced the same response. The alighting of a small insect on the same lily leaf was sufficient to cause the larva to dodge below. It is very probable that this response is responsible for the avoidance of many attacks by predaceous enemies.

Breathing Movements.—If a larva in the breathing position be watched carefully it will be noted that certain rhythmic movements are performed. These movements occur at intervals of from two to three seconds and appear to consist of an alternate contraction and expansion of the exposed region.

Extrusion of Excrement.—The voiding of excrement occurs in connection with the respiratory trips to the surface but since these trips are the direct effect of the demand for oxygen the extrusion of wastes is only an accompanying feature. The ratio of the number of respiratory trips to the number of extrusions is variable, depending upon the feeding activity of the larva. The following records will serve to illustrate the variation:

Larva no. 1	2 extrusions in 14 trips to surface.
Larva no. 2	1 extrusion in 11 trips to surface.
Larva no. 7	1 extrusion in 41 trips to surface.

LOCOMOTION.

Dissemination is accomplished in three distinct ways, viz., swimming, floating, and crawling.

Swimming.—The larva usually swims on the surface although when forced to do so it can swim to a limited extent below the surface. The specific gravity of the larva in all stages is very slightly less than that of water, thus making it easy to remain at the surface. Furthermore, the larva at all stages has an oily surface which prevents a "wetting" of the exterior and constitutes another means of remaining on the surface. This form of locomotion is accomplished by means of a series of rather vigorous horizontal undulations in the execution of which the whole body takes a part. The caterpillar lies in a trough in the surface which partially surrounds it and is propelled by the characteristic sinuous movements. The efficiency of this kind of locomotion varies with the age of the larva and the amount of disturbance of the surface of the water. Very young larvæ do not progress very rapidly but the full-grown ones are efficient swimmers, showing noteworthy speed and endurance. Larvæ, 4 cm. in length, were removed from their burrows and placed in the open water in a protected bay where the surface was perfectly quiet. They immediately began to swim and records were made of the rate of progression. In one instance a larva made a continuous swim of 250 feet in 19 minutes and at the end of the journey showed little or no signs of weakness. A number of similar records were made with similar results. Ripples, diminutive waves, and currents interfere seriously with this form of locomotion, even with full-grown larvæ, and little or no headway can be made against them. Owing to the fact that the situations which are favorable for the growth of their food plant are characterized by quiet water, the interference with dissemination due to surface disturbances is not great.

The advantage to the larva of this surface swimming is obvious. It must have free air and many of the journeys which it makes require a longer time than it can remain under water but the ability to swim on the surface makes it possible to secure air with ease.

When larvæ are placed beneath the surface of the water and released they immediately orient themselves in such a way that the head is towards the surface and, by means of the undulatory movements of the body, swim diagonally upward, continuing the effort until the surface is reached. Swimming below the surface is much less efficient than on the surface but it is usually effectual in assisting the larva in getting back to the top. When submerged they appear to lack the ability to do effective climbing on stems and other objects with which they come in contact.

When swimming on the surface the larva shows a strong tendency to climb upon any object which comes in its way and in case the object is immovable, has a smooth upper surface, is above the surface of the water, and broad enough to support the greater part of the length of the body, it will usually come to rest for a time. In cases where the object is very limited in size, as for example a stick, twig, or stem, the larva usually climbs over it and continues the journey. Larvæ may desert the leaves and voluntarily take to the water. When once in the water the swimming movements are usually kept up continuously until another flat, emergent object is reached. When a larva deserts one lily leaf the search for another food plant is entirely a random one and the contact with another leaf seems to be purely accidental. It merely keeps on swimming until it happens to choose a course which leads to a leaf. The larva apparently has no means of recognizing the presence of a food plant except when in contact with it. The writer observed instances where larvæ swam aimlessly about for a half hour without finding a food plant, having passed several times within a centimeter of a lily leaf.

The larvæ under observation showed a definite response to currents of low rapidity. When placed in the open water of a stream having a current of 10 feet per minute they almost invariably swam down stream. This accounts for the prevalence, at the mouth of Bessey Creek, of infested leaves showing attack only at the junction of the petiole with the leaf—an indication of the work of the older larvæ.

Floating.—Instances were observed where larvæ were transported from place to place by floating down stream on detached leaves and other floating objects. Occasionally a swimming larva became passive and, since it remained on the surface, it was carried along with the current.

Crawling.—Change of position on the leaf is accomplished by crawling and, in case lily leaves are contiguous, means is thus provided for the passage from one leaf to another. When in search of a new leaf the larva usually explores the periphery of the leaf, taking to the water and swimming only when it is not possible to reach another leaf from the supporting one. The explorations at the edge of the leaf consist in projecting one-third to one-half of the body over the edge and swinging it about as if seeking another support. If nothing is within reach, the body is withdrawn and the same performance repeated at another place. If, however, an object is within reach the larva immediately crawls upon it.

In cases where larvæ were deserting the old leaves it was not always possible to account for the departure. The two most common incentives apparently were (1) the presence of other larvæ on the same leaf, since only one larva can occupy the burrow in the petiole, and (2) the deterioration of the lily leaf and petiole which results in an unfitting of the plant for the larva.

ENEMIES.

These larvæ were eagerly snapped up by sunfish whenever there was opportunity. They were comparatively safe in the burrows but whenever they left the lily leaves and swam in open water the mortality was often high. Specimens removed from the petioles and thrown out into open water were soon discovered and captured. The undulatory swimming motion appeared to be fatally effectual in attracting the attention of fish. A number of instances were observed in which specimens thrown out were not molested so long as they remained motionless but unfortunately for them they remain quiet for only short intervals, often not exceeding 60 seconds, and soon after swimming began were snapped up. Fish of all sizes appeared to feed upon them. The larger fish always secured the prey at the first dash but small fish were observed to make three or four attempts before the full-grown larva could be secured.

The large water strider (*Gerris* sp.), common in the Douglas Lake region, was observed to attack the larvæ when they happened to be on the surface of the leaves. The ultimate effects of such an attack were not observed since in every case the larva attacked happened to be under observation for other data and the striders were driven off.

Department of Entomology,
Kansas State Agricultural College,
April 23, 1914.

LITERATURE CITED.

Beutenmüller, W.
'02 Descriptive Catalogue of the Noctuidæ found within Fifty Miles of New York City. Part II. Bull. Am. Mus. Nat. Hist., 16: 413–458. 4 pls.

Comstock, J. H.
'81 An Aquatic Noctuid Larva. Papilio, 1: 147–149.
'88 Aquatic Lepidopterous Larvæ. Am. Nat., 22: 468–469.

Grote, A. R.
'89 The Noctuidæ of North America and Europe compared. (Fourth Paper). Can. Ent., 21: 226–230.

Hampson, G.
'10 Catalogue of the Lepidoptera Phalænæ in the British Museum, 9: 260.

Miller, G. S. and Standley, P. C.
'12 The North American Species of Nymphæa. Contr. U. S. Nat. Herbarium, 16: 63–108. 12 pls., 40 figs.

EXPLANATION OF PLATE.

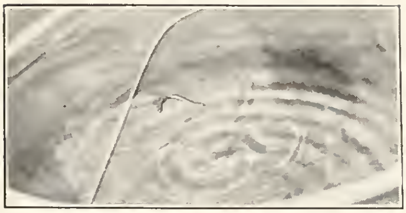
FIG. 1. Photograph of a nearly full-grown larva of *Bellura melanopyga* swimming on the surface of the water in the aquarium. The undulations of the body in performing the swimming movements are evident.

FIG. 2. Photograph of the same larva taken a few seconds later.

FIG. 3. Leaf of *Nymphæa americana* showing the character of the work of approximately mature larvæ. The radiating slits represent the work of the larvæ preliminary to the excavation of the petiole. Note the severe injury to the leaf in the region of the petiole. Leaves are sometimes almost completely severed from the petiole in this way.

FIG. 4. Leaves of *Nymphæa americana* showing the work of half- to full-grown larvæ and the characteristic form of the injury.

FIG. 5. Leaves of *Nymphæa americana* infested by half- to full-grown larvæ. The leaf near the center of the figure shows the absence of preliminary cutting in the form of radiating slits. Note on the same leaf the heap of excrement about the hole at the junction of leaf and petiole. This hole is the entrance to the burrow in the petiole.



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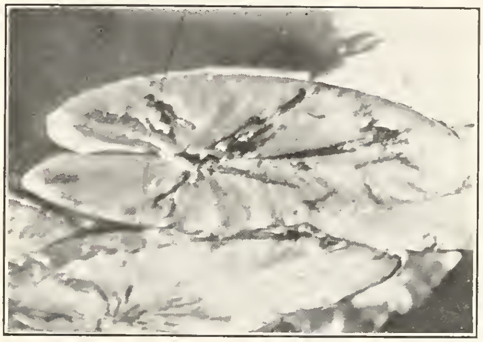

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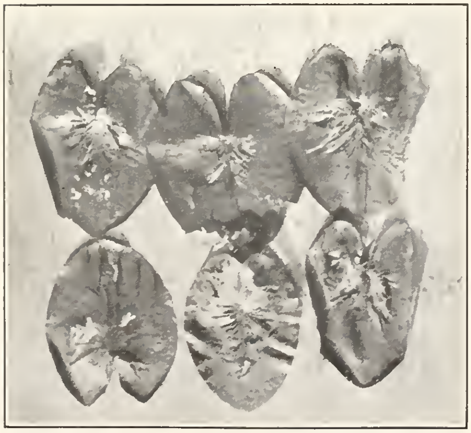

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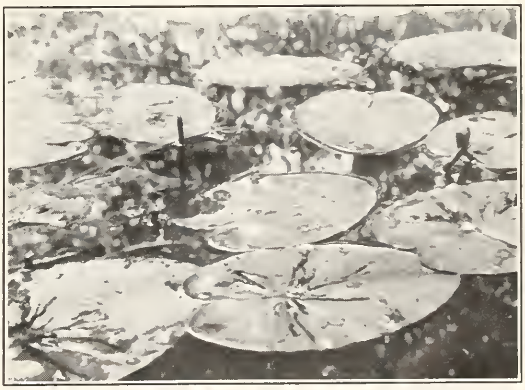

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FERTILIZATION IN THE PARASITIC COPEPOD, LERNÆOPODA EDWARDSII OLSSON.[1]

NATHAN FASTEN.

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The Female Reproductive Organs 117
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General Remarks.

Wright (1882), in discussing reproduction in the parasitic copepod *Achtheres micropropteri* deprecates the fact that so little is known of the exact method of fertilization in the parasitic copepods, and adds that "a thorough examination of the male reproductive apparatus of the Lernæopodidæ is very desirable for the purpose of elucidating the formation of the spermatophores in the parasitic Copepoda." Since Wright's work no one has published any results along this line. It, therefore, seemed advisable to investigate this problem a little more fully.

The following studies were made on the parasitic copepod *Lernæopoda edwardsii* Olsson which infests the gills of the brook trout *Salvelinus fontinalis*. The writer, while in the service of the Wisconsin Fish Commission, during the summer of 1912, secured a lot of fine stages of this organism in copulation, from the time the male attaches itself to the female until the actual fixation of the spermatophores near the genital pores of the female's body. Many mature males and females were also secured.

The material was fixed in a 5 per cent. solution of corrosive-acetic for eighteen hours and was then washed in running water for a day. Selected stages were stained in toto in borax-carmine for thirty-six hours, and the excess of stain was removed in water or in very weak acid-alcohol. The material was next passed through the various grades of alcohol, being left in each for about ten hours, and after being cleared in xylol was finally mounted in balsam.

---

[1] From the Zoölogical Laboratories, University of Wisconsin.

Many mature males, females, and copulating individuals were also imbedded in paraffine for sectioning. These were cut into frontal, sagittal and transverse sections, ranging from 3–6 μ in thickness, and stained either in Delafield's hæmatoxylin and eosin or in Heidenhain's iron-alum hæmatoxylin and acid fuchsin. The latter stains gave the finest results and were utilized almost exclusively.

The Male Reproductive Organs.

The male reproductive organs of *Lernæopoda edwardsii* are, in the main, similar to those of the free living copepods, as described by Gruber (1879). They are paired, and are located in the posterior half of the body (Figs. 1–2, *t.*, *v.d.*, and *sp.*). These organs lie in the space between the intestine and the lateral body wall, and consist of three main parts: (*a*) the testis (Figs. 1–2, *t*), (*b*) the coiled vas deferens (*v.d.*), and (*c*) the spermatophores (*sp.*). The testis is sac-like in appearance, with its anterior end projecting into a tube-like structure that ends blindly. When a section of the testis is examined under the microscope, three distinct zones can be recognized which correspond very closely to the three zones in the testis of *Diaptomus* sp., described by Ishikawa (1891). The anterior portion is the formative zone, and consists of spermatogonial stages. The middle region is the zone of growing spermatogonial cells, and lastly, the posterior region represents the maturation zone. Here the testis is in active proliferation and is filled with great numbers of spermatozoa. Each testis unites with the vas deferens posteriorly.

The vas deferens is an elongated coiled structure composed of three parts. The first portion of the vas deferens (Figs. 3–4, *v.d. 1*), lies nearest the lateral body wall of the copepod, and receives the spermatozoa from the testis. The second branch (*v.d. 2*), is a thin, tube-like structure that makes its way from the lower margin of the preceding portion (*v.d. 1*) and then coils up diagonally behind it, to a point slightly above the terminal region of the testis. This is best seen in Fig. 4. It then coils forward as the third lobe of the vas deferens (*v.d. 3*). This runs along posteriorly to a slit-like opening located in the extreme lower margin of the male's body. This opening is the ejaculatory pore (Figs. 3–6, *e.p.*), through which the spermatophore is extruded.

Each spermatophore (Figs. 1–6, *sp.*) originates in the third branch of the vas deferens. It is pear-shaped in form, and in the living organism is of a pale yellowish color. In Figs. 5–7 the structure can best be studied. The spermatogonial pouch, filled with spermatozoa (*sz.*), is enclosed by an outer wall of chitin (*ch.*). Immediately behind this there is a thin layer of cement-like substance (*c.*), that stains intensely black in Heidenhain's iron-alum hæmatoxylin. In the center of the spermatophore is a spherical pouch filled with a similar cement (*c.*) as is evident from the similarity of its staining reaction. This cement substance of the spermatophore appears to be continuous with the small sphere of cement found in the loop-like enlargement of the vas deferens (see Fig. 3, *v.d. 3*).

When the spermatozoa are mature, they are discharged into the first branch of the vas deferens. From here they pass into the second lobe, and finally, they migrate into the canal situated in the center of the third part of the vas deferens. This is shown in Fig. 5, where the canal (*can.*) conducts the spermatozoa into the spermatophoral pouch, located between the central sphere of cement and the outer cement wall. Here the sperm (Figs. 6–7, *sz.*) are stored until copulation occurs.

The Female Reproductive Organs.

The mature female is much bigger than the male, being about three times as long as the latter. Fig. 1 shows this size difference. The sex organs of the female lie dorso-laterally within the abdomen, between the intestine and the body wall. The ovaries (Figs. 1 and 9, *ov.*), are paired in character, and each gives rise to a slender oviduct (Figs. 1 and 9, *od.*), which makes its way around the intestine to the posterior portion of the body, where it opens to the exterior. Both oviducts unite slightly above their terminations into a broad pouch that later becomes the spermatheca and its accessories (Figs. 9 and 11, *s.*). From this pouch, two slender tubes are developed that open posteriorly through the genital pores (Figs. 1 and 8, *g.p.*). Also, within each oviduct, a small, spiral gland can be distinguished (Figs. 1 and 9, *c. g.*), which, under the microscope shows strong powers of refracting light. These structures later develop into the cement glands of the adult (Figs. 10–11, *c.g.*).

Fertilization.

About two and a half or three weeks after the attachment of *Lernæopoda edwardsii* to the brook-trout, the copeopod is ready to copulate. In two earlier papers (Fasten, 1912 and 1913), the attachment of the parasite to the host was discussed. As already stated, the mature male is very much smaller than the female. In order to fertilize the female, the male must release his hold on the gill of the brook-trout, and attach himself to the lower extremity of the female's body, in the neighborhood of the genital pores. This is accomplished in the following manner. When mature, the male, though still attached, makes circling movements with his body, thus coming in contact with a female. As soon as this occurs, the male clasps her with his maxillipeds, and at the same time, withdraws his second maxillæ from the filament of attachment to the gill. Then he moves towards the female's genital pores, and places himself in position for fertilization. The position assumed is shown in Fig. 1. These observations are similar to those made by Wilson (1911), on the copepod *Achtheres ambloplitis* Kellicott, infecting the rock bass.

When once in position, the male brings forward the posterior region of his body to a position near the genital pores. The spermatophores are then ejected through the ejaculatory pores, and by the aid of his free maxillae the male attaches them near the genital openings of the female (see Fig. 8). The cement found in the spermatophores is the substance that makes them adhere tightly. As soon as they are attached, the spermatozoa wander through the genital pores and are conducted into the spermatheca, where they are stored until the eggs are mature for fertilization. When the spermatozoa have all migrated into the spermatheca, the spermatophores collapse, and become transparent, shell-like, yellowish spheres (Figs. 9–10, *sp.*).

The female may be fertilized more than once. As many as six spermatophores were found clinging to the genital pores of some of the females, showing that these females have, in all probability, been fertilized three times. After fertilization, the male drops off from the female's body and dies. The female however, grows enormously in size. During this growth period, the ovaries develop and produce a great many eggs. The cement gland also develops completely, and from each side of the extreme lower margin of the abdomen, an egg-sac grows out. The oviducts communicate with these egg-sacs directly. Figure 10 shows the abdomen of an adult female, with the eggs (*o.*), the oviducts (*od.*), the cement glands (*c.g.*), the spermatheca (*s*)., the spermatophores (*sp.*), and the egg-sacs (*e.s.*).

The eggs are discharged into the oviducts, and are then passed down posteriorly, where they are fertilized by the spermatozoa which are stored in the spermatheca. Then they are coated with a layer of cement from the cement glands, and finally pass into the egg-sacs, where they develop into larvæ. The cement coating hardens into one of the egg covers. Fig. 11 shows the relation of the spermatheca (*s.*) to the oviducts (*od.*). The spermatheca is filled with great numbers of ripe spermatozoa.

These observations on the structure of the reproductive apparatus of the adult female corroborate those of Miculicich (1905), who worked with Lernæopodidæ of the genus *Brachiella*.

I wish to express my thanks to Professors M. F. Guyer and A. S. Pearse for reading this paper and for their helpful suggestions.

SUMMARY.

1. About two and a half or three weeks after the attachment of *Lernæopoda edwardsii* to the brook-trout, the parasite is mature for fertilization.

2. The mature male is about one third as long as the mature female. The reproductive organs of the male are paired, and are located in the posterior region of the body, between the intestine and the body wall. They consist of a testis, a coiled vas deferens, and a spermatophore.

3. The female reproductive apparatus is also paired in character and is located within the abdomen, between the intestine and the body wall. The ovaries give rise to slender oviducts, which open at the lower extremity of the abdomen. Slightly

above their terminations, the oviducts combine to form the spermatheca, which opens to the exterior through the two genital pores. Within each oviduct a spiral gland can be seen, which later develops into the cement gland.

4. In order to effect fertilization, the male makes circling movements while still attached to the host, thereby meeting a female. Then the male releases his hold on the host, attaches himself to the female, and moves down posteriorly in the vicinity of the genital pores.

5. The male bends his abdomen upward towards the female, and extrudes the spermatophores from the reproductive organs. These he manipulates with his second maxillæ and soon attaches them about the genital pores of the female.

6. The spermatozoa wander from the spermatophores into the spermatheca of the female and are stored until the eggs are ripe enough to undergo fertilization.

7. The eggs pass down the oviducts when mature, and are fertilized by the stored spermatozoa as they pass the spermatheca. The embryos pass into the external egg-sacs, where they develop into larvæ.

BIBLIOGRAPHY.

Fasten, N.

'12 The Brook Trout Disease at Wild Rose and Other Hatcheries. (The Brook Trout Disease in Wisconsin Waters.) Rep't. Wis. Fish Com., 1911–1912, pp. 12–22.

'13 The Behavior of a Parasitic Copepod, *Lernæopoda edwardsii* Olsson. Jour. of An. Beh., Vol. 3, pp. 36–40.

Gruber, A.

'79 Beiträge zur Kenntniss der Geschlechtsorgane der freilebenden Copepoden. Zeitschr. wiss. Zool., Bd. 32, pp. 407–442.

Ishikawa, C.

'91 Studies on the Reproductive Elements. I. Spematogenesis, Ovagenesis, and Fertilization in *Diaptomus sp.* Jour. of Col. of Sc., Imp. Univ., Japan, Vol. 5, Pt. I., pp. 1–34.

Miculicich, M.

'05 Zur Kenntniss der Gattung Brachiella Cuv. und der Orginisation der Lernæopodiden. Zool. Anz., Bd. 28, pp. 599–620.

Wilson, C. B.

'11 North American Parasitic Copepods. Part 9. The Lernæopodidæ. Proc. U. S. Nat. Mus., Vol. 39, pp. 189–226.

Wright, R. R.

'82 Notes on Parasitic Copepods, No. I. Proc. of Canad. Inst., Vol. I., pp. 243–254.

EXPLANATION OF PLATES.

All drawings were made with the aid of the camera-lucida. The magnification is given after the description of each figure.

Abbreviations.

a.f. = attachment filament.
ant. 1 = first antennæ.
ant. 2 = second antennæ.
b. = brain.
c. = cement-like substance of spermatophores.
can. = canal of v. d. 3.
c. g. = cement gland.
ch. = chitinous covering of spermatophore.
d. g. = digestive gland.
e. p. = ejaculatory pore.
e. s. = egg-sacs.
f. g. = frontal gland.
g. = gill.
g. p. = genital pores.
i. = intestine.
m. = mouth.
mx. 2 = second maxillæ.
mx. g. = maxillary gland.
mxp. = maxillipeds.
mxp. g. = maxillipedal gland.
o. = eggs.
od. = oviduct.
ov. = ovary.
s. = spermatheca.
s. g. = shell gland.
sp. = spermatophores.
sz. = spermatozoa.
t. = testis.
v. d. = vas deferens.
v. d. 1 = first branch of vas deferens.
v. d. 2 = second branch of vas deferens.
v. d. 3 = third branch of vas deferens.

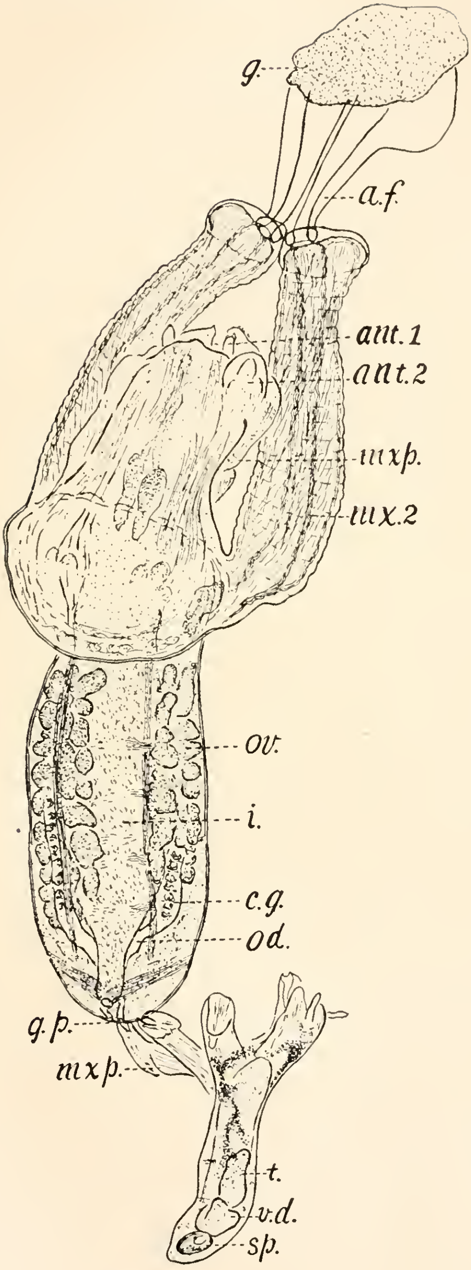
Plate I.

Explanation of Figures.

1. Male and female copepods in position for copulation. The female is attached to the gill (*g.*) by the funnel-like attachment filament (*a. f.*). The male is attached to the female near the genital pores (*g. p.*). × 57.

2. Side view of a mature male showing the position of the reproductive organs (*t.*, *v. d.*, and *sp.*). × 120.

3. Enlarged view of the reproductive organs of the male shown in Fig. 2. × 218.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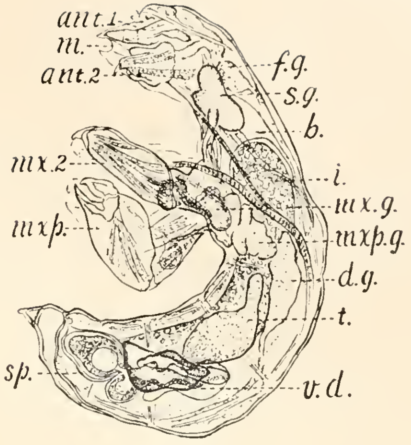
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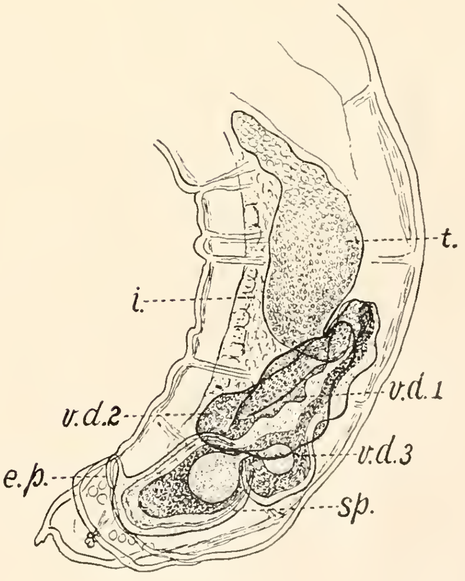
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NATHAN FASTEN.

Plate II.

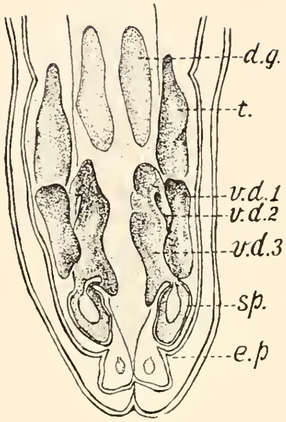
Explanation of Figures.

4. Dorsal view of the posterior extremity of a mature male, showing the position of the various parts of the reproductive organs. × 144.

5. Sagittal section through the third lobe of the vas deferens and the spermatophore. The canal-like tube (*can.*), that conducts the spermatozoa is seen in *v.d. 3*. × 560.

6. Frontal-section through the spermatophores, showing their structure. × 800.

7. Cross-section through a spermatophore slightly above its central region, showing its structure. × 1,240.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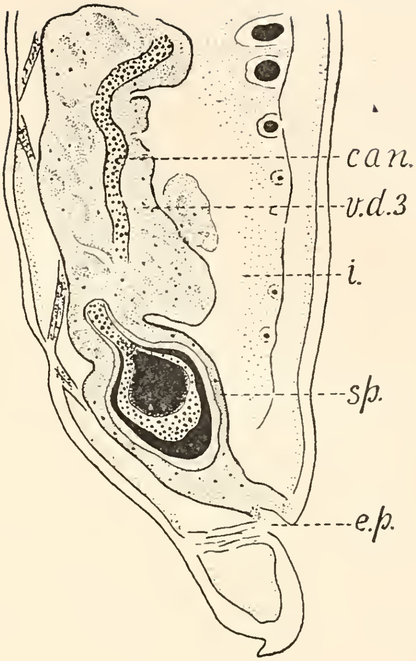
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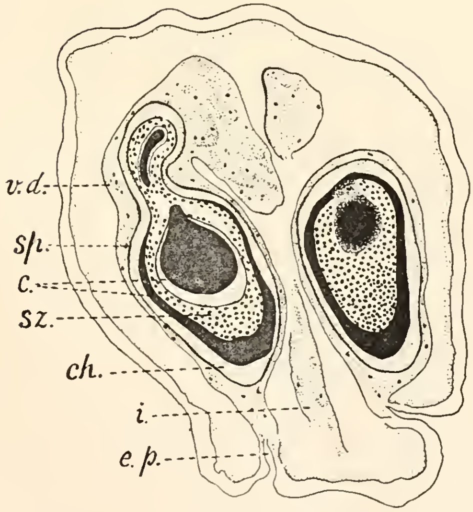
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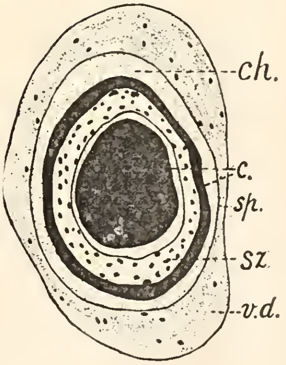

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NATHAN FASTEN.

Plate III.

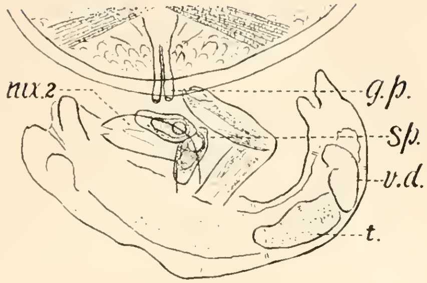
Explanation of Figures.

8. The process of fertilization. The male is here seen manipulating the extruded spermatophores (*sp.*) between his second maxillæ (*mx. 2*), in the vicinity of the genital pores (*g. p.*) of the female's body. × 100.

9. Side view of a young female after fertilization. The spermatophores (*sp.*), are attached near the genital pores. × 76.

10. Ventral view of the abdomen of an adult female showing the relation of the reproductive organs to each other. × 57.

11. Cross-section through the abdomen of an adult female in the region of the spermatheca. × 76.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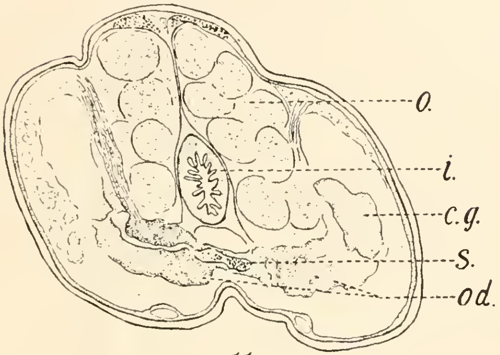

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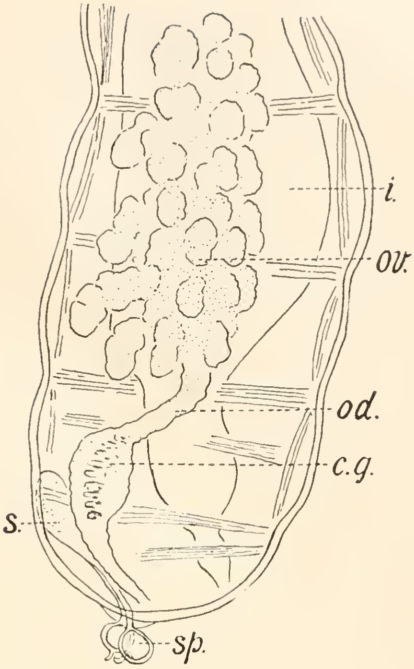
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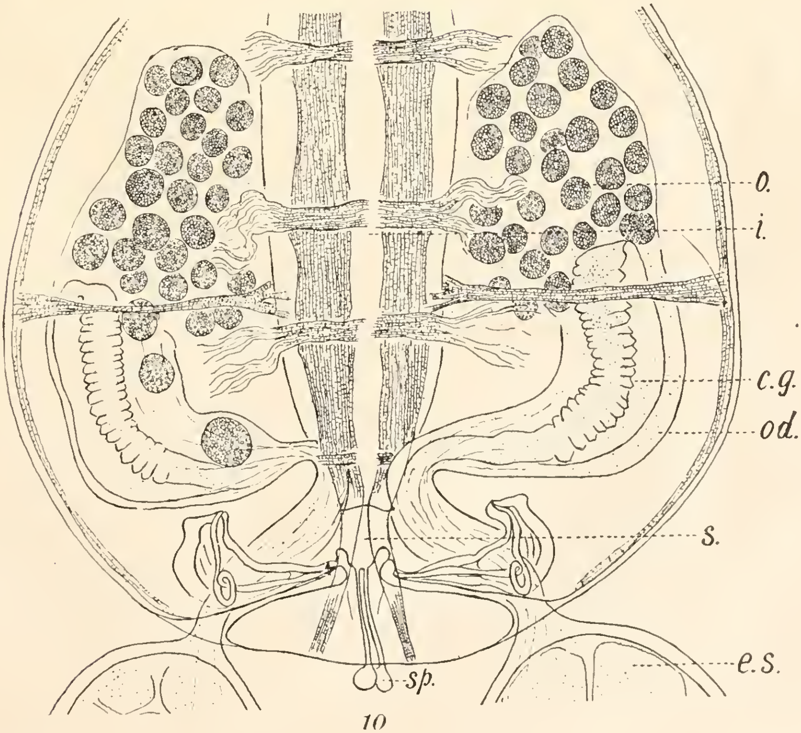
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Vol. XXVII. September, 1914. No. 3

BIOLOGICAL BULLETIN

THE FOOD AND FEEDING HABITS OF FRESHWATER MUSSELS.[1]

WILLIAM RAY ALLEN.

THE FEEDING HABITS OF MUSSELS.

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INTRODUCTORY.

With the increasing commercial demand for mussels and the rapidly diminishing supply we shall have need for all the available information concerning their life history. Lefevre and Curtis ('12) have done a great deal toward solving the problems which beset artificial glochidial infection. Much may still be learned concerning the practicability of artificial feeding, the nature of their enemies and of their diseases.[2]

---

[1] This paper is the result of observations made at the Indiana University Biological Station, at Winona Lake, Indiana, under the direction of Prof. Will Scott. It forms contribution No. 130 of the Zoölogical Laboratory of Indiana University.

[2] An important obstacle to their successful cultivation is offered by the pollution of streams. Searches which I made on several occasions in a certain section of the Mississinewa river, into which oil and salt water from oil wells, and sewage from a paper mill are poured, failed to produce a single living mussel, although there was an abundance of empty shells. At this point, during the dry season, great schools of fish take refuge in the shallow tributaries which are freer from pollution.

The mussel, regardless of its considerable size, depends entirely for food on the microscopic organisms floating in the water, and offering little resistance to capture. That this diet is sufficient, is probably due to the comparatively inactive life of the animal. Energy is further economized by a partial combination of the functions of respiration and food-getting in the same organ—the gill.

The Cilia and their Action.

The filaments of the gills are covered with cilia which intercept the particles contained in the water and prevent their passing through the gills with the water. They become entangled in mucus and through the action of these cilia such particles are wafted toward the mouth in streams. If they are of a harmless nature or of food value, they are permitted to enter the alimentary tract. During the incubation of the glochidia, the female gives up a greater or less part of one or both of the gills for marsupial purposes. At this period these parts are of little use for respiration or for the collection of food.

Cilia similar to those of the gills line the entire branchial chamber, cover all organs which come into contact with the water, and also line the alimentary tract. They are, as is always true of cilia, in constant motion during life; they act independently of nervous control and in a single plane. Their concerted action is in the form of waves—resembling in appearance the passing of a breeze over a field of grain, or, as Field ('09) has said, the movement of a bank of oars. The direction which these waves or streams take varies in the several organs. But all of the streams taken together are coördinated to accomplish a certain common end. So vigorous and powerful are the cilia that, when an organ or portion of one is removed and placed upon a smooth surface for study, the whole mass is moved by their action slowly and steadily in a direction opposite to that in which they are directed. Small parts will even climb the side of a watch glass. McAlpine ('88) found that seven twelfths by weight of the soft part of the animal may thus be moved by ciliary action. As long as the part so removed is kept in water their activity continues, even for several days. In one instance McAlpine kept the cilia of marine clams living for eight days after their removal from the animal. This illustrates the fact that, while the cilia generate considerable power, they accomplish it upon a minimum of metabolism.

The general character of the feeding habits of mussels has been known for nearly a century. Fragmentary bits of information on the various phases of the subject are to be found in the Molluscan literature, particularly that concerning marine forms, to whose study economic interest has added impetus.

Scope and Nature of Investigation.

It has been the purpose of this investigation to determine for fresh water forms, (1) what their food is, (2) how food material reaches the alimentary tract. (For the most part there is great similarity to the marine lamellibrancha in these two particulars, but there are a few essential differences.) (3) Incidental to the above a few observations concerning digestion.

Material.

In the work upon the nature of the food, lake-dwelling mussels were used altogether. In the other studies river forms were also used. Besides one or two unidentified species, the following were employed:

Lampsilis luteolus Lamarck
Lampsilis subrostratus Say
Quadrula rubiginosa Lea
Lampsilis alatus Say
Lampsilis rectus Lamarck
Unio gibbosus Barnes
Lampsilis ligamentinus Lamarck
Anodonta grandis Say

Being by far the most numerous at Winona Lake, *L. luteolus* was used for the greater part of the work.

Anatomy, and Previous Work on the Function of the Cilia.

The mouth of the lamellibranch lies nearly as far as possible from the external openings, just behind the anterior adductor muscle. It is thus well protected from the entrance of harmful substances. It is flanked above and below by the thin narrow lips. The upper lip is continuous with the outer labial palp on each side, while the lower lip is prolonged into the inner right and left palps. Most of the ciliary currents of the contiguous faces of the palps and of the lips are directed forward to the mouth (Figs. 3 and 5). The outer or non-contiguous faces of both palps and lips (Fig. 4) as well as the edge of the inner face of the lips, bear cilia which are directed backward and away from the mouth. Thus particles which find their way between the palps are carried to the mouth. As will soon be seen, very little undesirable matter ever reaches the mouth or palps, but even here Wallengren ('05) has pointed out how selection and rejection may be made.

As shown diagrammatically in Fig. 4, the inner surface of the labial palps, except their outer margins, are made up of minute vertical ridges, or furrows. These constitute a quite complex mechanism for the sorting of material. In cross section (lengthwise of the labial palps) they appear as in Fig. 5.

Upon the ridges as elsewhere occurs a ciliated epithelium. But the ciliary currents are disposed in a unique manner. Upon the anterior slope of each ridge they are directed backward (Fig. 5, *p*) while those on the posterior slope lead forward (Fig. 5, *a*). This seeming conflict is not such in fact, because only one set of cilia comes into action at a time. The position of the ridges determines which set shall function at a given moment. Their normal position seems to be that seen in the two ridges on the right in Fig. 5, a somewhat reclining one, overlapping one another toward the anterior. Thus the after slopes (*a*) are ordinarily brought uppermost, the ciliary currents leading to the mouth are upon the surface, while the cilia (*p*) which lead from the mouth lie somehwat underneath the ridges. So long as no adverse stimuli are received, particles which lie between the palps are thought to be passed on forward from one ridge to another, to the lips and mouth.

In the event that distasteful matter reaches the palps a reflex erection of the ridges (Fig. 5, *I*) brings uppermost the cilia leading backward (*p*) and such material is returned from summit to summit to the edge of the palps and discharged into the mantle chamber.

It is extremely difficult to observe the cilia which lie at the bottoms of the furrows (*mf*). Wallengren (*l.c.*) ascribes to them the duty of carrying lengthwise of the furrow to the lower margin of the palps the minute particles that may fall between the ridges. But Siebert ('13) thinks they lead in the opposite direction.

In the event that any particles get past the palps they may still be rejected at the mouth. A strong compression of the lips will force them outward to the edge of the lips, where they encounter the cilia directed backward (Figs. 3 and 4, lower margin) and are carried to the edge of the palps and dropped into the mantle chamber.

The outer surfaces of the palps and lips have as their function the removal of particles from the mantle chamber (Fig. 4).

McAlpine's (*l.c.*) observations upon the movements of detached parts led him to conclude that the palps and gills have nothing to do with feeding, and that they are concerned only with carrying away foreign material. But Wallengren's (*l.c.*) conclusions are based upon far more careful and logical experiments, and Siebert's (*l.c.*) recent paper on the epithelium of Anodonta is of a confirmatory nature.

Observations.

The ciliary currents may be observed quite readily on a mussel from which the shell and mantle of one side have been removed, or on detached parts, which, as stated, continue to exhibit ciliary activity for a long time. Small quantities of carmine, indigo, or other nearly neutral coloring matter may be dropped upon the part to be studied, and their behavior noted. Care must be exercised in the amount of water used. The less water the better, within limits, for in a large amount of water currents may obscure the action of the cilia. The surface of the organ must be level in order to offset gravitational disturbance. A small piece of any ciliated organ, when placed in a watch glass with water and a very little color, will show under low power both the cilia and their currents in great detail.

The Ciliary Streams.

The figures will show more clearly than description the course of the streams of material collected from the water. All the ciliary currents of the inner gills (Figs. 1, 2, 4, 6, and 7) are seen to be directed downward. When they reach the lower edge of the gill they pass around the points of the lamellæ to the under side. Here the lamellæ of the two faces of the gill form an inverted trough (Fig. 7, *tr*) in which the particles small enough to be used as food may be carried to a point just above the labial palps (Figs. 1 and 2, *X*). At this point they accumulate in strings of mucus until their weight causes them to fall into the mantle chamber, or into the trough formed by the upper margins of the palps. This depends upon the desirability of the material for food, or rather upon the presence or absence of unfavorable stimuli, and is probably regulated by reflexes, since the palps are quite motile, even after being severed.

The ciliation of the outer gills tends upward (Figs. 1 and 6). When particles carried upward by their inner surfaces reach the top, they are passed over to the inner gill, and thence to the point described above. But the gleanings obtained by the outer surface of the outer gill pass over to the mantle at the line of their attachment (Figs. 1 and 6). All material transmitted by the outer gill and collected by the uppermost part of the mantle is removed forward by the latter to a point just above the attachment of the palps to the mantle and body wall. So long as the upper edge of the palps remains applied to the mantle this material passes into the trough between the palps, backward around the line of attachment of the two palps, then forward again between them (Figs. 3 and 4). In case of an unfavorable stimulus the palps are withdrawn from the mantle at this point the material glides past them and is carried downward and backward by the cilia of the lower part of the mantle to the region of the excurrent siphon (Fig. 1). All the cilia of the lower part of the mantle are directed toward the posterior. So are those of the epithelium of the lower body wall. Their function is that of collecting particles to be thrown out. A small portion of the body wall, near the upper margin, is ciliated like the same portion of the mantle, and must be concerned with the collection of food.

From the foregoing account and from the figures it is evident that the region of the labial palps is the center toward which all the ciliation of the upper part of the mantle chamber tends. All material gathered by both gills and by the dorsal part of the body epithelium and mantle must finally reach one of the three adjacent points—(1) the mantle just above the palps, (2) the body wall just opposite the first point, or (3) a point on the edge of the inner gill just above the labial palps. All these are within easy reach of the palps (Figs. 1, 2, 4, and 6).

No one, to my knowledge, has succeeded in inducing a mussel to behave normally, after the shock of removing parts of the shell and mantle in order to observe the palps at work. But I have repeatedly obtained the reactions which occur. When the palps lie in contact with either body, mantle, or gill, their collections of material pass between the palps and mouthward. Otherwise such material is carried on down by the several structures and discarded. The fact that the upper margins of the labial palps adhere to each other and form a trough (Figs. 4 and 6) makes it possible to reach at least two of the three sources of supply simultaneously.

Since we have the mechanism for such a method, and since the reactions, though fragmentary and under abnormal conditions, are of a confirmatory nature, we may safely infer that the labial palps do actually accept or refuse food, either through reflex stimuli or in response to volition.

The Function of the Mucus.

The entire epithelium touching the branchial chamber is abundantly supplied with glands which secrete a mucous substance (Siebert, *l.c.*). The mucus envelops and binds together in strands the material to be transported by the cilia. This is particularly true of those particles which are of a very distasteful nature. That this secretion is dependent on local reflexes is quite evident from the fact that it may be stimulated in an organ entirely severed.

It is this collection of food in a film of mucus, which makes possible the mechanism of the furrowed surface of the labial palps. If each particle were manipulated independently, it would tend to eddy back and forward between the opposing streams of cilia, and considerable confusion might result. But a strand of material spans the summits of several ridges, and while touching cilia that lead in both directions, it obeys the ciliary streams which lie uppermost and exert the greater force upon it.

Conclusions on the Function of the Ciliary Currents.

The surfaces of the gills and of the upper part of the mantle, and the contiguous faces of the labial palps, in fact, nearly all the upper parts of the mantle chamber, have for their general purpose the carrying of food to the mouth. The lower part of the mantle chamber, upon which the heavier fragments are likely to fall, are concerned principally with removing undesirable matter from the animal.

The Selection and Rejection of Food Particles.

Observers have differed widely in their notions of the ability of the mussel to select its food. To me it is evident that there are, to summarize, four points where such choice is exercised:

(1) The labial palps, at the upper margin.
(2) The labial palps, on the furrowed surfaces.
(3) The mouth.
(4) The incurrent siphon.

As to the last, it is surrounded by a row of pointed, fleshy papillæ, having a resemblance to plant structures. These have two sensory functions—tactile and gustatory; for upon being disturbed mechanically they are withdrawn into the shell, while a continued teasing, or a strong chemical stimulus results in the closing of the shell, or perhaps only the siphons.

It is true that some material of no food value finds its way into the alimentary canal. But the quantity is far smaller than if no selection were made, and is of a harmless nature. All distinctly injurious substances are rigidly excluded.

That which has been rejected at the mouth, palps, or gill accumulates upon the lower posterior margin of the mantle or body wall, along with the collections made by these parts themselves, and is here massed in clots of mucus. When this has attained considerable size the animal ejects it with a rapid current of water, set in motion by a quick contraction of the adductor muscles and closing of the shell (Figs. 1 and 2, *r*).

The Rate of Siphoning.

An effort was made to determine the rate at which water is siphoned through the mussel. If this can be done it will contribute to several quantitative studies relative to the feeding habits, and that of the effect of temperature and other conditions upon the activity of the cilia.

But it is very difficult to attach apparatus for making measurements to the siphons of the animal. Intimate contact must be made to avoid leakage and a high per cent. of error. Such contact irritates the mussel, so that it does not behave normally.

In only one mussel did I succeed in obtaining what seemed a normal circulation of water, when under this annoyance. This was done by placing a short piece of soft rubber tubing in the excurrent siphon. Into the end of this was thrust a calibrated glass tube, having a capacity of 2 c.c. between two given marks. The point of a pipette containing neutral coloring matter was thrust into the rubber just outside the siphon. The mussel with this simple apparatus was put into an aquarium near the lake, where the water could be changed frequently and the lake conditions maintained. A touch upon the pipette released a drop of coloring matter into the tube, where it encountered the stream flowing from the excurrent siphon.

This individual was a *L. luteolus* weighing 200 grams with the mantle chamber filled. It required five seconds for the pigment to pass between the two marks upon the tube, whenever the incurrent siphon was opened fully. The reading was repeated a number of times at intervals, with the same result.

While these are but meager results, they give at least an idea of the volume of water siphoned. At the above rate there are siphoned 24 c.c. per minute, 1,440 c.c. per hour, or 34,560 c.c. per day. To filter a liter of water would require 42 minutes.

Digestion.

Digestion fluctuates more in the case of mussels than does their feeding. That is, the animal continues to feed regardless of appetite; but the degree with which the food so ingested is really made use of seems to depend upon the relation of supply and demand. At times nearly all the intestinal contents are found to be at least partially digested; while again much material is found, even in the rectum, in perfect preservation, and often the fæces themselves contain forms which are apparently unaffected. Hence we may conclude that appetite fulfills its function by the control of the secretion of the digestive juices, without the voluntary regulation of the food supply.

The Crystalline Style.

The literature on the lamellibranchs is particularly rich in speculations concerning the function of the crystalline style; but I cannot forbear adding a word here parenthetically on the subject, by calling attention to the excellent work done by Mitra ('01) upon it. He reviews all the previous theories and repeats the experiments, extends them, and brings physiology, chemistry, and comparative morphology to bear upon the matter, so that there seems to be no way of escaping his conclusion that the style is a digestive ferment which converts starch into sugar. The previous hypothesis of Gegenbaur, that it is a secretion of enteric epithelium, he holds to be true, but says that this does not account for its existence. Against Balfour's notion that the style is a rudiment of a radular sac he brings six weighty proofs, and dismisses with two objections each the theories of Claus and Sedgwick, that it is an excretion product and a reserve of nutriment, respectively.

In two minor particulars my observations do not agree with those of Mitra. (1) In an experiment upon the renewal of the style he concludes that it appears and disappears *periodically*. But from the description of his experiment we find that the water and food supply were renewed at regular intervals, whence the periodicity. In a similar experiment of my own the crystalline style was found to disappear only with the lack of food, and to be regenerated only when food was supplied, regardless of time. Then too, as long as the food supply is abundant the style is never wanting. In all mussels freshly removed from the lake it was found to exist. In all mussel freshly removed from the lake it was found to exist. In these matters the work of Haseloff ('88) also confirms my point.

Hence the food supply must be a factor in its secretion. As it is dissolved when food is lacking, and as it is a proteid, why is not Sedgwick's theory as to its being a reserve food supply also true?

(2) The presence in the core of the crystalline style of cells similar to those found in the liver epithelium leads Mitra to conclude that the liver is the probable origin of the structure. This also does not seem well substantiated, especially in species where the style is found in a diverticulum. No channel for the passage of a secretion from the liver to the seat of the style has been discovered, and the ciliation of the stomach and intestine forbids their aiding in its transmission.

Since the publication of the above paper Grave ('03) has suggested in his work on the oyster that the crystalline style may perform the duty of preventing coarse particles from passing through the digestive canal. In fresh water mussels I can see but one way in which the style may attend to that function—by digesting the said particles. There are at least two objections to this explanation for fresh water mussels (1) As we have seen, the animal is well protected against the entrance of such particles. (2) In case they were admitted to the stomach but kept out of the intestine, they would accumulate in the stomach, for it is not equipped with either a muscular or a ciliary system by which these could be expelled through the mouth. Then too, the mouth is no larger than the intestine and no more capable of receiving them. The largest body I have seen in the alimentary tract was a fragment of *Oscillatoria* (or similar form) measuring 1.5 mm. in length.

Food Materials.

No one but Zacharias ('07) seems to have undertaken a detailed examination of the contents of the alimentary tract of the freshwater forms, so I give the results of a purely qualitative examination. In marine mussels it is said that the food consists almost altogether of minute plant forms, and of these almost all are Diatomaceæ. In the mussels which I examined I found a somewhat different condition. In the first place there is a little higher proportion of animal food present. Living animals are found but rarely, and most of these are apparently living transiently upon the contents of the tract. But the mussel does not refuse minute dead animals or small fragments of sloughed and decaying animal tissue. In many cases bits of material seemingly the carapaces of small crustacea are found to resist digestion throughout the alimentary canal. However most of the animal matter consists of shapeless unidentifiable fragments.

In order to determine the ability of mussels to capture and digest living animals, a few were kept without food for several days, then a rich culture of *Paramecium* was added. This was found to be of sufficient nutrient value to regenerate the crystalline style in part. The digestive tract was seen to be filled again with half assimilated material. Few living *Paramecia* were discovered beyond the stomach, thus corroborating Vogt and Jung's statement ('94) that digestion is nearly completed in that organ.

In the second place, the plant material does not consist so largely of diatoms, there being probably as many other algal forms. Very few desmids were observed, in fact only three genera which I could identify with certainty. But Winona Lake does not produce many desmids, and it is not probable that they are discriminated against where they exist more abundantly.

The following is a list of genera recognized. There were several more which I could not identify.

DIATOMACEÆ.

- *Amphora*
- *Arachnoidiscus*
- *Cocconeis*
- *Cocconema*
- *Coscinodiscus*
- *Craspedodiscus*
- *Cymbella*
- *Epithemia*
- *Fragilaria*
- *Gomphonema*
- *Melosira*
- *Navicula*
- *Pleurosigma*
- *Surirella*

OTHER ALGÆ.

- *Anabæna*
- *Aphanocapsa*
- *Cœlastrum*[1]
- *Cylindrocapsa*
- *Eudorina*[1]
- *Glœocystis*
- *Leptothrix*
- *Lyngbya*
- *Merismopedia*[1]
- *Œdogonium*[1]
- *Oscillatoria*
- *Pandorina*[1]
- *Pediastrum*[1]
- *Protococcus*

- *Synedra*
- *Triceratium*

DESMIDACEÆ.
- *Closterium*
- *Netrium*
- *Staurastrum*[1]

- *Rhaphidium*
- *Scenedesmus*
- *Spirogyra*
- *Tetraspora*
- *Ulothrix*
- *Vaucheria*

MISCELLANEOUS CONTENTS.

- Inorganic fragments,
- Plant and animal debris,
- Mold,
- Ova and spermatozoa
  - Of other animals,
  - Of the same individual or species,
    - (The sperm living and in motion),
- Spores and swarm spores.

The posterior half of the mussel shell, the part protruding above the substratum, is usually very richly encrusted with diatoms and other algae. This may serve as a private garden, and particles dislodged by the passage of the animal along the bottom thus be brought into the incurrent siphon.

In addition to the very valuable assistance which I have received from Prof. Scott, I wish to acknowledge by indebtedness to the John Crerar Library of Chicago, through which I was able to procure several useful references, and to Profs. C. H. Eigenmann and W. C. Curtis, who have made indispensible critical suggestions.

BIBLIOGRAPHY.

Call, R. E.

'99 The Mollusca of Indiana. 24th An. Rept. Ind. Dept. Geol. and Nat. Res., pp. 335–535.

Conn, H. W., and Webster, L. W.

'08 A Preliminary Report on the Algæ of the Fresh Waters of Connecticut. Conn. State Geol. and Nat. Hist. Surv., pp. 1–78.

Coupin, H.

'93 Sur l'élimination des matières étrangères chez les Acéphales. Compt. Rend. de l'Acad. des Sci. Paris, CXVII., pp. 373–376.

Field, I. A.

'09 The Food Value of Sea Mussels. Bull. U. S. Bur. Fish., XXIX., pp. 81–128.

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[1] Also noted by Zacharias (*l. c.*).

Gary, L. R.

'07 The Cultivation of Oysters in Louisiana. Bull. No. 8, Gulf Biol. Sta., Baton Rouge, pp. 1–56.

Grave, C.

'03 Investigations for the Promotion of the Oyster Industry of North Carolina. Rept. U. S. Fish Comm., 247–327.

Haseloff, B.

'88 Ueber den Krystallstiel der Muscheln. Kiel. Inaug. Diss. Osterode, Abstr. Biol. Cent., VII., pp. 683–684. Abstr. J. R. Micr. Soc. (London), 1888, p. 566. Abstr. Am. Nat., XXII., p. 936.

Headlee, T. J., and Simonton, J.

'03 Ecological Notes on the Mussels of Winona Lake. Proc. Ind. Acad. Sci., pp. 173–179.

Kellog, J. L.

'00 The Ciliary Mechanism in the Branchial Chamber of the Pelecypoda. Science (2), XI., pp. 173–173.

Lefevre, G., and Curtis, W. C.

'12 Studies on the Reproduction and Artificial Propagation of Freshwater Mussels. Bull. U. S. Bur. Fish., XXX., pp. 103–201.

McAlpine, D.

'88 Observations on the Movements of Detached Parts in Bivalve Mollusks. Trans. Roy. Soc. Victoria, XXIV., pp. 131–149.

'88 Observations on the Movements of the Entire Detached Animal, and of Detached Parts of Bivalve Mollusks. Proc. Roy. Soc. Edinburgh, XV., pp. 173–204.

Mitra, S. B.

'01 The Crystalline Style of the Lamellibranchiata. Quart. Jr. Micr. Sci., XLIV., pp. 592–602, Pl. XLII.

Moore, H. F.

'08 Volumetric Studies of Food and Feeding of Oysters. Bull. U. S. Bur. Fish., XXVIII., pp. 1293–1308.

Ortmann, A. E.

'12 Notes upon the Families and Genera of the Najades. An. Carnegie Mus., VIII., No. 2, pp. 221–365.

Posner, C.

'75 Ueber den Bau der Najadenkieme. Bonn.

Siebert, W.

'13 Das Körperepithel von Anodonta cellenis. Zeitsch. f. Wiss. Zoöl., CVI., pp. 449–526.

Simpson, C. T.

'00 Synopsis of the Naiades, or Pearly Freshwater-Mussels. Proc. U. S. Mus., 22, pp. 501–1044.

Stenta, M.

'02 Zur Kenntniss der Strömungen im Mantelraume der Lamellibranchiaten. Arb. Zoöl. Inst. Wien, (T.) XIV., pp. 211–240. Contains a good historical bibliography.

Vogt, C. et Jung, E.

'94 Traité d'Anatomie Comparée, Vol. I., Acéphales.

Wallengren, H.

'05 Zur Biologie der Muscheln. I. Die Wasserströmungen. Acta Univ. Lunds., Ser. 2, Bd. 1, No. 2, pp. 1–64. II. Die Nahrungsaufnahme. Ib., No. 3, pp. 1–59.

Williams, L. W.

'07 The Structure of Cilia, Especially in Gastropods. Am. Nat., XLI., pp. 545–551.

Wolle, F.

'87 The Freshwater Algæ of the United States.

'92 The Desmids of the United States.

'94 The Diatomaceæ of North America. Comenius Press, Bethlehem, Pa.

Zacharias, O.

'07 Planktonalgen als Molluskennahrung. Arch. f. Hydrobiologie und Planktonkunde, Stuttgart, II, pp. 358–361.

EXPLANATION OF PLATE I.

The arrows indicate the directions of the ciliary currents.

The arrows which follow the margins of the inner gills show the direction of ciliary currents in the troughs which form their under edges (Fig. 7, *tr*).

x x x x x x indicates the line which divides mouthward-flowing streams from those leading posteriorly.

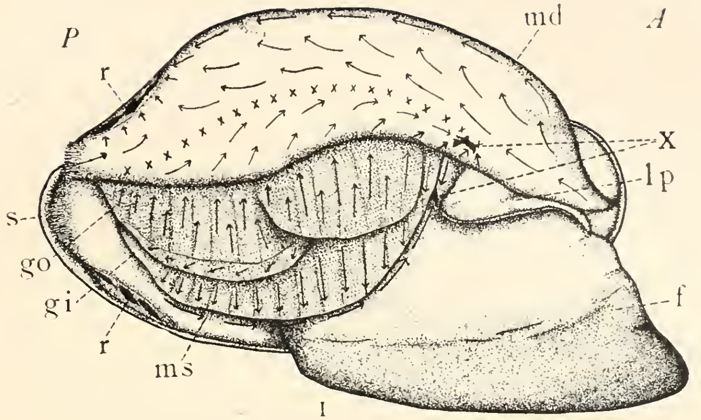
In all except Figs. 6 and 7 the left side is posterior and the right anterior.

Fig. 1. (Adapted from Wallengren.) The organs concerned with food-getting; the right shell removed and mantle folded back; showing ciliary streams of mantle and gills.

Fig. 2. (Adapted from Wallengren.) As in Fig. 1; mantle gills and palps of right side folded back; showing ciliary streams of inner gill and body wall.

Abbreviations.

A, anterior.
f, foot,
gi, inner gill,
go, outer gill,
lp, labial palps,
md, right mantle,
ms, left mantle,
P, posterior,
r, mucous accumulations of refuse material,
s, siphon,
X, point of convergence of ciliary currents near labial palps.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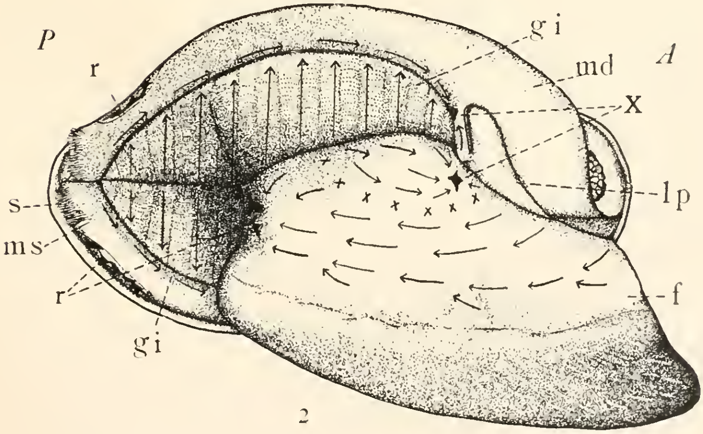
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EXPLANATION OF PLATE II

Fig. 3. The inner labial palp; outer palp removed; normal ciliary streams of furrowed surface.

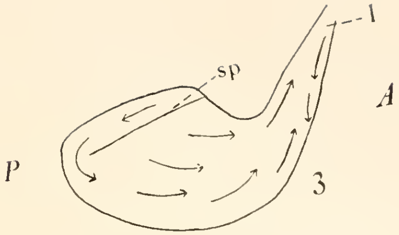
Fig. 4*a*. (Slightly diagrammatic. See Fig. 4*b*.) The left labial palps, adjacent mantle surface, and end of gills; right mantle, gills and palps, and whole body removed; showing point (*X*) where streams of food from gills and mantle converge at the labial palps.

Broken arrows indicate the ciliary currents of the mantle; solid arrows those of the gills; and barbed arrows those of the palps. Where the broken arrows are seen upon the gills they are meant to apply to the mantle just beneath.

Fig. 4*b*. Key to Fig. 4*a*; all organs removed except left mantle, gills and palps; the area within double lines has been enlarged as Fig. 4*a*.

Abbreviations.

A, anterior,
aa, anterior adductor muscle,
fs, furrowed surface of labial palps,
gi, inner gill,
go, outer gill,
l, lips
lp, labial palps,
lv, liver,
ma, unfurrowed margin of palps,
ms, left mantle,
P, posterior,
pa, posterior adductor muscle,
r, mucous accumulations of refuse material,
sc, suprabranchial chamber,
se, excurrent siphon,
si, incurrent siphon,
sp, line of attachment of the palps to each other,
X, point of convergence of ciliary currents near labial palps.



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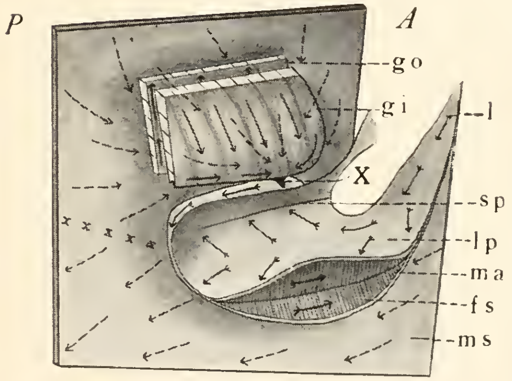
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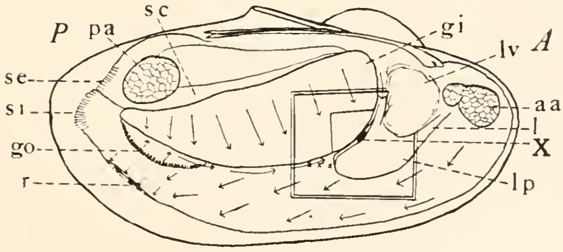
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EXPLANATION OF PLATE III.

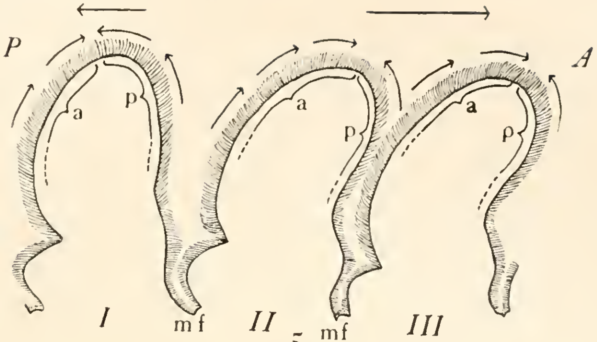
FIG. 5. Cross section of ridges on contiguous surfaces of labial palps (Fig. 4*a*, *fs*) showing their several positions. While *a* is uppermost (as in *II* and *III*) material is carried mouthward; when *p* is raised by the erection of the ridge (as in *I*) the stream is reversed toward the posterior, and the cilia *a* no longer function.

FIG. 6. (Slightly diagrammatic.) Cross section of the ciliated organs concerned with food collecting; showing how the labial palps (*lp*) by occupying several positions in the mantle chamber, may or may not receive the material collected at *X*. As a matter of fact, the palps can span almost the entire width of the mantle chamber at this point, but the width is exaggerated here to show details with greater clearness.

FIG. 7. (After Posner.) Cross section of the edge of inner gill; showing the groove (*tr*) by which material is transported toward the palps.

Abbreviations.

I, ridge erected; current reversed,
II–III, normal position of ridges,
A, anterior,
a, cilia directed anteriorly,
f, foot,
gi, inner gill,
go, outer gill,
ic, interlamellar chamber,
lp, labial palps,
m, mantle.
mf, cilia at bottom of furrows,
P, posterior,
p, cilia directed posteriorly,
r, mucous accumulations of refuse material,
tr, trough at margin of inner gill,
X, points of convergence of ciliary currents near labial palps.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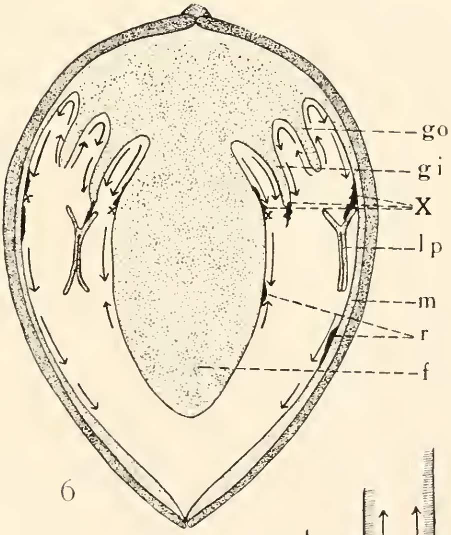
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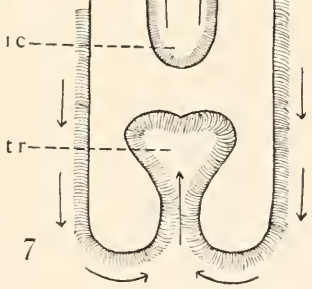


7

W. R. ALLEN

THE MATURATION DIVISIONS IN ASCARIS INCURVA.

H. B. GOODRICH.

In the following preliminary account of the spermatogenesis and ovogenesis of a nematode, *Ascaris incurva* Rud., it is intended to call particular attention to a remarkable XY-group that forms the extreme case thus far observed. The X-group consists of no less than eight components of which one is a vestigial microsome, while the Y is represented by but a single chromosome. The nearest approach to this case is that described by Edwards, '10, for *Ascaris lumbricoides*, where in the heteropolar mitosis the X-element is found to consist of five components unmated by a Y. The extreme example hitherto described in which a Y exists is that of *Acholla multispinosa* described by Payne, '10, in which case five X-components are opposed by one Y, but here the Y is equal to or larger in mass than the combined X-elements.

Ascaris incurva is a parasite found in the stomach of the swordfish, *Xiphias gladius* L. The material was collected at Woods Hole during the summer of 1913, and most of this thus far studied was fixed in strong Flemming's or Gilson-Carnoy's fluid.

A study of the spermatogonial cells has proved unsatisfactory as the chromosomes are closely massed and the cytoplasm stains deeply. Counts vary from 33 to 35. Fig. 1, showing a cell somewhat over-extracted as is desirable to give the necessary contrast, gives a count of 35 chromosomes including the microsome.

During the growth stages a part of the chromatin is massed in a large irregular karyosome. Late prophase or metaphase figures of the first spermatocyte division show 21 chromosomes or 22 if the Y is widely separated from its mate in the X-group (Fig. 2).

Early anaphase figures of the first spermatocyte division show most clearly the unequal nature of the separation of the chromosome groups. Thirteen autosomes lying at or near the periphery of the plate divide equally, thus forming two anaphase plates of thirteen chromosomes, typically arranged in a ring except that at a point of one daughter plate a gap is observed, opposite which in the other plate is a fourteenth chromosome (Figs. 4 and 6). There remain eight chromosomes lagging in the center of the spindle and arranged in a characteristic plate consisting of six chromosomes of the average size, the microsome and a larger long chromosome arranged in an approximately oval or circular plate with the long chromosome projecting from the periphery (Figs. 5 and 8). As the daughter plates separate, this peculiar group tips, apparently as a unit, so that the long chromosome approaches the gap in the ring of thirteen autosomes. Eventually this whole group passes to the center of the ring and thus the two daughter-cells (second spermatocytes) receive respectively 14 and 21 chromosomes. Size relations and position facilitate the identification of homologous daughter chromosomes of the anaphase plates when these are observed superimposed within a single section (Figs. 4 and 6). Thus the thirteen autosomes of either daughter ring may be readily identified, and, by elimination, the fourteenth of one ring unmated in the other. This fourteenth chromosome must therefore be considered as a Y-chromosome mated by that member of the X-group, the long chromosome, which is first inserted into the gap of the one ring, corresponding to the space occupied by the fourteenth chromosome of the other. Side views of metaphase figures of this division (Fig. 3) show the Y-chromosome lying opposite one end of its longer mate to which it may be united. Fig. 3 is an optical section of such a group showing the Y, its long mate, six other elements of the X-group massed and undivided, surrounded by certain of the dividing autosomes. A cleft in the X-chromosomes indicating the line of division in the ensuing second spermatocyte division may often be observed in the anaphase stage of the first division (Fig. 8). The long chromosome splits lengthwise and usually the chromatin appears concentrated at either end of each half giving a quadrivalent appearance and suggesting a tendency to separate in two parts, one to remain the mate of the Y and the other to increase the number of those unmated.

From the foregoing it will be clear that the secondary spermatocyte divisions should be of two classes, one showing 21 chromosomes including the microsome, and the other 14. Examination of numerous metaphase plates has proved this in the clearest manner to be the case (Figs. 9 and 10). This condition may be compared with that in *Ascaris lumbricoides* in which the two classes of secondary spermatocyte cells show respectively 19 and 24 chromosomes, or with *Acholla multispinosa* in which the spermatids receive either 11 or 15 chromosomes.

Oogonial cells showing division figures have not been found but the constant presence of 21 chromosomes in the maturation divisions of the egg indicates most certainly that the diploid number in the female is 42. Metaphase and anaphase plates of the first oöcyte division repeatedly give the count of 21 chromosomes. Figs. 11 and 12 show two daughter plates found superimposed within a single section and each gives the count of 21 chromosomes including the microsome. Such an observation of the dividing microsome, together with its constant behavior as a member of the X-group in the spermatocyte cells, gives the conviction that this minute body is in reality a chromosome. The second oöcyte plates (Fig. 13) again reveal the expected count of 21 chromosomes of which one is the microsome. Side views of both oöcyte anaphases show a clean separation of daughter plates with no sign of lagging chromosomes, so conspicuous in the first spermatocyte division.

These results demonstrate that in *Ascaris incurva* there are formed two classes of spermatozoa, one bearing 21 chromosomes, the other 14 chromosomes; and they indicate that fertilization of the egg carrying 21 chromosomes by a spermatozoön of the first class gives rise to the females which have 42 chromosomes and by one of the second class to the males which have 35 chromosomes. This cycle of the chromosomes may be summarized in the following formulae in which the autosomes are designated as A and the sex chromosomes as X and Y.

Spermatozoa of Two Classes.		Egg.		Gamete.
$13A + 8X$	+	$13A + 8X$	=	$26A + 16X = 42$ (female).
$13A + Y$	+	$13A + 8X$	=	$26A + 8X + Y = 35$ (male).

I would like to express my thanks to Dr. Edwin Linton for advice and aid in obtaining material and to Dr. E. B. Wilson at whose suggestion and under whose guidance the work has been undertaken.

Columbia University,
May, 1914.

REFERENCES.

Edwards, C. L.

'10 Idiochromosomes in *Ascaris megalocephala* and *Ascaris lumbricoides.* Archiv für Zellforschung, Bd. 5.

Payne, Fernandus.

'10 The chromosomes of *Acholla multispinosa.* Biol. Bull., Vol. 18, March.

Explanation of Plate.

All figures are drawn with a Zeiss 1.5 mm. apochromatic objective, a no. 18 compensating ocular and projected with camera-lucida to table level. Figures as here reproduced give a magnification of 3,000 diameters. Figs. 1, 2, 3, 7, 8, 11, 12, 13 are from material fixed in Gilson-Carnoy's fluid and Figs. 4, 5, 6, 9, 10 from material fixed in strong Flemming's fluid.

Fig. 1. Spermatogonial metaphase.

Fig. 2. First spermatocyte metaphase showing the XY group centrally located and surrounded by 13 autosomes.

Fig. 3. First spermatocyte metaphase. An optical section of a side view showing the Y-chromosome opposite the end of the long X-chromosome, six other X-elements and three pairs of dividing autosomes.

Figs. 4, 5, 6. First spermatocyte anaphase. Figures are from one spindle; Fig. 4 showing upper ring of 13 autosomes and Y, Fig. 6 showing lower plate of 13 autosomes and gap opposite position of Y in Fig. 4, Fig. 5 showing the intervening X-element of 8 chromosomes.

Fig. 7. First spermatocyte anaphase. A side view of late stage showing daughter plates of autosomes and X-group viewed edgewise.

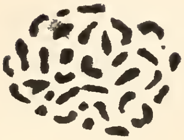
Fig. 8. First spermatogonial anaphase. Late stage showing 5 autosomes from upper plate, 3 from lower and the X-group of 8 chromosomes.

Fig. 9. Second spermatocyte metaphase showing 14 chromosomes.

Fig. 10. Second spermatocyte metaphase showing 21 chromosomes.

Figs. 11, 12. First oocyte anaphase. Daughter plates from one spindle and each showing 21 chromosomes.

Fig. 13. Second oocyte metaphase showing 21 chromosomes.



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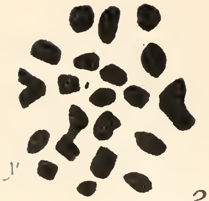

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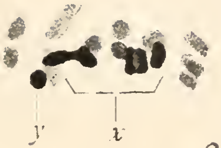

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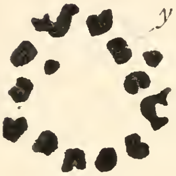

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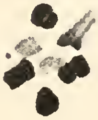

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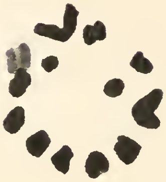

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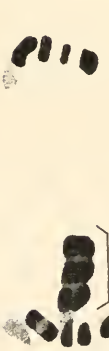

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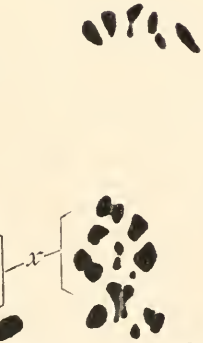

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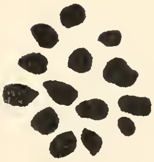

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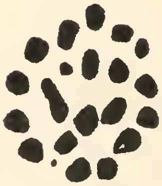

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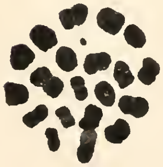

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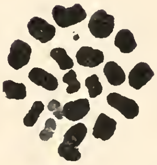

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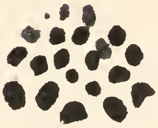

13

LINKAGE OF THE FACTOR FOR BIFID WING. THE BIFID WING AND OTHER SEX-LINKED FACTORS IN DROSOPHILA.

ROBERT CHAMBERS, JR.

The experiments described in the following pages were made primarily in order to test whether crossing-over of factors has any subsequent effect on the linkage relations of the factors involved. For instance, if a red eyed fly with bifid wings is crossed to a white eyed fly with normal wings there will appear in F_2 the non-crossover classes, red bifid and white normal, and also some crossovers white bifid and red normal. These cross-overs (white bifid) were then used to determine whether the same linkage values would reappear in their grandchildren; in other words, whether a crossover in a particular place predisposes to more frequent crossing-over in the same place.

I wish to take this opportunity of acknowledging my indebtedness to Professor Morgan and to Mr. Bridges for their advice and suggestions.

The term "bifid" was given to a type of wing in which the second inner vein fails to reach the margin, often producing a bifid or forked wing.[1] The forked condition is variable. A constant feature, however, which the forked condition frequently accompanies, is a fusing of the wing veins at the base of the wing. Flies possessing this characteristic cannot fly.

The following abbreviations are used in the tables to denote the factors taken account of in the flies studied:

For eye color—R = red, V = vermilion, W = white;
For body color—Gr = Gray, Y = yellow;
For wing shape—L = long, Bf = bifid;
X = factor to which sex linked factors are linked;
o = gamete not possessing X-factor.

The factors R, V, Gr and Bf are in association with the X-factor which is duplex in the female and simplex in the male.

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[1] Morgan, *Science*, Vol. 35, March 12, 1912.

For the sake of simplicity these factors will be considered in this paper as single units.

EXPERIMENT I.

A long-winged vermilion eyed female was mated with a bifid winged red eyed male. The results to be expected in the F^1 and F^2 generations are shown in Table I.

TABLE I.

TO ILLUSTRATE EXPERIMENT I.

P^1	LVX − LVX = LV♀	BfRX − o = BfR♂
	P^1 Eggs LVX	Sperm BfRX, o
F^1	LVX − BfRX = LR♀	LVX − o = LV♂
	F_1 Eggs { (1) LVX, (2) BfRX, (3) LRX, (4) BfVX,	Sperm { LVX, o
F^2	LVX − LVX BfVX − LVX } = LV♀	LVX − o = LV♂ BfRX − o = BfR♂
	BfRX − LVX LRX − LVX } = LR♀	LRX − o = LR♂ BfVX − o = BfV♂

The F^1 generation consisted of LR females and LV males. The actual results of the F^2 generation are shown in Table II.

TABLE II.

RESULTS OF EXPERIMENT I.

F^2	♀		♂				Total ♂♂	Per Cent. of Crossovers.
	LR	LV	BfR	LV	BfV	LR		
	273	256	123	144	50	62	379	30

All the combinations expected are realized. Owing, however, to the coupling in pairs of the P^1 factors, the numbers of males in the two classes consisting of the combinations LR and BfV are fewer than those of the other two classes. That they appear at all is evidence of the incompleteness of the linkage, the new combinations being due to a rearrangement or crossing over of factors within the germ cells of the F^1 flies. The strength of linkage may be determined by that percentage of the total which are crossovers, in this case 112/379 or approximately 30 per cent.

On mating the F^2 males possessing the new combination BfV with normal red eyed long winged flies (LR) we obtain LR male and female offspring. When these are interbred we obtain the F^2 results shown in Table III.

TABLE III.

RESULTS OF EXPERIMENT I., CROSSOVER.

F^2	♀	♂				Total ♂♂	Per Cent. of Crossovers.
	LR	BfV	LR	LV	BfR		
	498	80	123	71	53	327	38

These results even with comparatively small numbers show that the new combination BfV produced by a crossing over of factors possesses approximately the same strength of coupling as did the original.

EXPERIMENT II.

In the reciprocal cross, BfR females with LV males, the offspring consist of LR females and BfR males. In the second generation the four classes of males occur in the same proportionate numbers as those in Experiment I.

The results to be expected are shown in Table IV.

TABLE IV.

TO ILLUSTRATE EXPERIMENT II.

P^1 BfRX – BfRX = BfR ♀ LVX – o = LV♂
P^1 Eggs BfRX Sperm LVX, o

F^1 BfRX – LVX = LR ♀ BfRX – o = BfR♂

$$F_1\ \text{Eggs}\begin{cases}(1)\ \text{BfRX}, & (2)\ \text{LVX},\\ (3)\ \text{BfVX}, & (4)\ \text{LRX},\end{cases}\quad \text{Sperm}\begin{cases}\text{BfRX, o}\\ \end{cases}$$

$$F^2\quad \left.\begin{array}{l}\text{BfRX} - \text{BfRX}\\ \text{BfVX} - \text{BfRX}\end{array}\right\} = \text{BfR}♂ \qquad \begin{array}{l}\text{BfRX} - \text{o} = \text{BfR}♂\\ \text{LVX} - \text{o} = \text{LV}♂\end{array}$$

$$\left.\begin{array}{l}\text{LVX} - \text{BfRX}\\ \text{LRX} - \text{BfRX}\end{array}\right\} = \text{LR}♀ \qquad \begin{array}{l}\text{BfVX} - \text{o} = \text{BfV}♂\\ \text{LRX} - \text{o} = \text{LR}♂\end{array}$$

The actual results of the experiment are shown in Table V.

TABLE V.

RESULTS OF EXPERIMENT II.

F^2	♀		♂				Total ♂♂.	Per Cent. of Crossovers.
	LR	BfR	LV	BfR	LR	BfV		
	103	108	57	85	31	33	206	31

EXPERIMENT III.

Experiment III. confirms the results of Experiment I. LV females were crossed with BfR males. The F^1 females, all of which possess the gametic constitution, LVX–BfRX, (see Table I.), instead of being allowed to breed with their F^1 brothers, were mated with normal wild males possessing the gametic constitution RLX–o. The results are shown in Table VI. Although the cultures show a rather wide range of fluctuation it is doubtful if any of these are significantly different from 32, which is the value given by the total.

TABLE VI.

RESULTS OF EXPERIMENT III.

LV♀ × BfR♂

F^1 LR♀♀ and LV♂♂

LR♀ of F^1 × pure LR♂

F^2		♀	♂				Per Cent. of Crossovers.
Bottle No.	Transfer.	LR	LV	BfR	LR	BfV	
1	a	185	70	44	18	13	
	b	120	37	33	18	15	
	c	72	12	24	8	12	
		377	119	101	44	40	28
2	a	133	40	39	20	16	
	b	93	34	26	18	11	
		226	74	64	38	27	32
3	a	150	50	50	22	18	
	b	97	33	31	14	13	
		247	83	81	36	31	29
4	a	156	50	39	19	17	
	b	105	32	30	22	13	
		261	82	69	41	30	32
5	a	118	26	26	19	13	
	b	94	21	21	15	16	
	—	212	47	47	34	29	40
6	—	155	71	27	16	15	24
7	—	181	58	42	21	22	30
8	—	199	51	58	30	28	35
9	—	112	21	16	15	9	39
Totals		1970	606	485	275	231	32

EXPERIMENT IV.

LY females were mated with BfGr males. The F^1 generation consisted of LGr females and LY males. Interbreeding these gave rise to the four possible classes of males as shown in Table VII.

TABLE VII.

TO ILLUSTRATE EXPERIMENT IV.

P^1 LYX – LYX = LY♀ BfGrX – o = BfGr♂
Gametes LYX BfGrX, o

F^1 LYX – BfGrX = LGr♀ LYX – o = LY♂
F$_1$ Eggs { (1) LYX, (2) BfGrX, (3) LGrX, (4) BfYX Sperm { LYX, o

F^2
LYX – LYX }
BfYX – LYX } ± LY♀
LGrX – LYX }
BrGrX – LYX } = LGr ♀
LYX – o = LY♂
BfGrX – o = BfGr♂
LGrX – o = LGr♂
BfYX – o = BfY♂

The actual results of the experiment are shown in Table VIII.

TABLE VIII.

RESULTS OF EXPERIMENT IV.

P^1 LY♀ × BfGr♂

F^1 LGr♀ × LY♂

F^2	♀		♂				Per Cent. of Crossovers.
Bottle No.	LGr	LY	BfGr	LY	BfY	LGr	
1	69	29	46	41	0	7	
2	53	43	34	23	1	1	
3	70	59	57	46	5	1	
4	93	71	77	76	2	4	
Totals	285	202	214	186	8	13	5

Table VIII. shows that the linkage of the factors for bifid and yellow is stronger than for bifid and vermilion studied in Experiment I., the per cent. of crossovers being 5.

On mating males of the crossover class BfY with pure LR females we find in the F^2 generation (Table IX.) that the new combination persists in the same percentage as did the original combinations depicted in Table VIII. These results are in harmony with those obtained in Experiment I.

TABLE IX.

RESULTS OF EXPERIMENT IV. THE RECIPROCAL CROSS.

P1	BfGr ♀ × LY ♂
F1	LR ♀ × LR ♂

F2	♀	♂				Per Cent. of Crossovers.
Bottle No.	LGr	LGr	BfY	LY	BfGr	
5	126	67	53	3	5	
6	237	110	99	7	3	
7	102	50	37	2	1	
8	174	60	59	2	2	
Total	649	287	248	14	11	4.5

EXPERIMENT V.

This experiment is a repetition, during the following summer, of Experiment IV. The results of both reciprocal crosses are given in Tables X. and XI.

In order to secure large numbers the parent flies were transferred to fresh bottles every ten days. The transfers are indicated in the second column.

TABLE X.

RESULTS OF EXPERIMENT V. REPETITION OF EXPERIMENT IV. (Cf. TABLE VIII.)

F2		♀		♂				Per Cent. of Cross-overs.
Bottle No.	Trans-fer.	LGr	LY	BfGr	LY	BfY	LGr	
1	a	27	26	35	20	4	3	
	b	96	84	82	81	1	1	
		123	110	117	101	5	4	
2	a	54	51	48	42	4	1	
	b	45	31	33	26	4	3	
	c	61	49	45	36	1	4	
		160	131	126	104	9	8	
3	a	113	113	93	79	10	4	
	b	167	150	149	114	8	7	
		280	263	242	193	18	11	
4	a	19	32	18	16	1	1	
	b	78	66	75	73	7	4	
	c	232	69	198	64	6	9	
	d	97	12	77	11	—	7	
		426	179	368	164	14	21	
Totals		989	683	853	562	46	44	6

The results of the reciprocal cross are shown in Table XI.

TABLE XI.

RESULTS OF EXPERIMENT V. THE RECIPROCAL CROSS. (Cf. TABLE IX.)

F²		♀		♂				Per Cent. of Cross-overs.
Bottle No.	Trans-fer.	LGr	BfGr	LY	BfGr	LGr	BfY	
5	a	33	38	24	30	2	3	
	b	88	51	77	73	6	5	
	c	59	40	36	33	1	0	
	d	55	37	43	46	5	5	
		235	166	180	182	14	13	
6		194	146	111	148	16	5	
7		75	39	50	53	0	4	
8		121	114	78	82	4	4	
9		104	96	40	69	4	4	
Total		729	561	459	534	38	30	6

The percentage of crossovers is the same as that shown in Table X. and slightly larger than that in the corresponding experiment in Table IX.

EXPERIMENT VI.

LW females were mated with BfR males.

The LR females and LW males of the F¹ generation were allowed to interbreed. Table XII gives the numbers produced in the resulting F² generation. The percentage of crossovers is approximately 8.

TABLE XII.

RESULTS OF EXPERIMENT VI.

LW♀ × BfR♂

F¹ LR♀ × LW♂

F²	♀		♂				Per Cent. of Crossovers.
Bottle No.	LR	LW	LW	BfR	LR	BfW	
1	218	182	194	181	16	12	7
2	94	85	100	103	15	15	13
3	115	86	69	77	2	5	5
Total	427	353	363	361	33	32	8

The strength of coupling of the new combinations in the crossovers was determined by mating the crossover BfW males with pure LR♂. Table XIII. gives the results of this mating.

Table XIII.

Results of Experiment VI.- Crossovers.

LR♀ × BfW♂

F² LR♀ × LR♂

F²	♀	♂				Per Cent. of Crossovers.
Bottle No.	LR	LR	BfW	LW	BfR	
1	329	144	111	11	12	7
2	394	166	145	15	15	9
3	193	96	64	5	7	7
4	438	173	147	21	18	11
5	152	74	51	7	8	11
6	301	106	58	6	6	7
Total	1807	759	576	65	66	9

The crossing back of the crossovers occurs in approximately the same percentage as did the crossovers to the original combinations in Experiment VII.

The results here harmonize with those of Experiment I. We may legitimately infer that the large classes in the F² generation are those with the combinations occurring in the grandparents no matter whether the grandparents have acquired those factors early or late in their phylogenesis.

Experiment VII.

The results of the reciprocal cross, BfR females by LW males is shown in Table XIV.

Table XIV.

Results of Experiment VII. Reciprocal of Experiment VI.

BfR♀ × LW♂

F¹ LR♀ × BfR♂

F²		♀		♂				Per Cent. of Cross-overs.
Bottle No.	Trans-fer.	LR	BfR	LW	BfR	LR	BfW	
1	a	161	99	108	105	6	7	
	b	135	77	100	87	4	6	
	c	18	10	17	14	0	1	
		314	186	225	206	13	14	6
2		76	54	85	65	3	4	4
3		117	91	86	83	6	4	6
4		166	136	130	146	5	3	3
5		157	119	113	129	3	2	2
6		174	142	113	120	5	3	3
7		111	80	81	83	2	3	3
8		120	75	64	94	3	7	6
9		143	117	107	107	5	4	4
Total		1378	1000	1004	1033	45	44	4

The number of crossovers is far too small to give the same ratio found in Experiment VI. Here the percentage is only 4.

EXPERIMENT VIII.

This is a modification of Experiment VI. LR females were mated with BfW males. The F^1 generation consisted of LR males and females. The LR ♀ were removed from their brothers and crossed with pure BfW males. This was done in order to secure four possible classes, in the F^2 generation, not only of males but also of females thus rendering the female counts also available for study.

Table XV. shows the gametic constitution of the flies used and the combinations expected in the F^1 and F^2 generations.

TABLE XV.

TO ILLUSTRATE EXPERIMENT VIII.

P^1	LRX – LRX = LR ♀	BfWX – o = BfR ♂
	Gametes LRX	BfWX, o
F^1	LRX – BfWX = LR ♀	LRX – o = LR ♂
	LR ♀ of F^1 × BfW ♂ (from stock)	
	F_1 Eggs (1) LRX, (2) BfWX, (3) LWX, (4) BfRX	Sperm BfWX. o
F^2	LRX – BfWX = LR ♀	LRX – o = LR ♂
	BfWX – BfWX = BfW ♀	BfWX – o = BfW ♂
	LWX – BfWX = LW ♀	LWX – o = LW ♂
	BfRX – BfWX = BfR ♀	BfRX – o = BfR ♂

In Table XVI. are given the actual results of the experiment. Judging from the total numbers of the F^2 flies the break in the coupling occurs in the ratio of 5 per cent. in both females and males. Only the normal range of variations from this are apparent when the numbers from individual bottles are considered.[1]

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[1] In the previous experiments 4–6 females were placed with as many males in the same bottle. For Experiments VIII. and IX. one female with 2–3 males was placed in a bottle. She and the males were transferred every 6–8 days. Some of the females lasted for six weeks by which time they were fairly exhausted of eggs.

TABLE XVI.

RESULTS OF EXPERIMENT VIII.

LR♀ × BfW♂

F^1 LR♂

LR♀ of F^1 × BfW♂ (from stock bottle).

F^2		♀					♂				
Bottle No.	Transfer.	LR	BfW	LW	BfR	Per Cent. of Crossovers.	LR	BfW	LW	BfR	Per Cent. of Crossovers.
1	a	45	30	3	2		36	35	3	1	
	b	39	26	4	1		56	26	1	4	
	c	83	83	5	7		66	92	3	5	
	d	63	53	1	6		61	76	7	4	
	e	15	12	0	0		17	13	2	1	
	f	2	1	0	0		1	0	0	0	
		247	205	13	16	6	237	242	16	15	6
2	a	40	19	0	2		35	34	0	2	
	b	45	55	3	1		50	44	3	3	
	c	66	75	4	1		67	75	2	3	
	d	51	46	5	4		48	39	3	2	
	e	33	39	2	1		32	34	4	2	
		235	234	14	9	5	232	226	12	12	5
3	a	52	35	4	1		29	47	4	1	
	b	20	24	3	0		29	23	1	1	
	c	34	28	4	1		44	25	0	3	
	d	29	22	2	1		23	29	0	1	
	e	10	11	3	0		8	12	2	1	
		145	120	16	3	7	133	136	7	7	5
4	a	88	90	6	3		94	102	10	1	
	b	105	101	8	4		90	122	4	5	
	c	54	38	0	2		59	49	3	3	
	d	28	37	7	3		32	33	2	3	
	e	23	12	1	1		17	14	2	1	
		298	278	22	13	6	292	320	21	13	5
5	a	57	70	4	1		59	71	1	3	
	b	38	33	4	1		38	41	0	2	
	c	24	33	1	0		23	24	0	2	
	d	19	18	1	0		14	14	1	2	
		138	154	10	2	4	134	150	2	9	4
6		103	73	7	6	7	97	79	6	4	5
7		38	30	0	2	3	56	45	2	3	5
8		120	93	8	7	7	90	125	6	4	4
9		209	190	5	5	2	185	169	13	14	7
10		88	66	5	1	4	61	79	7	3	7
11		63	60	5	3	6	95	77	5	7	7
12		162	143	8	5	4	149	129	9	11	7
13		162	141	8	7	5	170	159	7	8	4
14		156	138	10	6	5	196	157	12	7	5
15		139	116	5	4	3	97	111	6	4	5
16		181	155	13	5	7	171	154	8	9	5
Total		2322	2053	141	89	5	2246	2229	130	119	5

Experiment IX.

For this experiment both males and females with crossed over factors were used. These were taken from the F^2 generation produced in Experiment VIII. On looking over Table XV. it will be noticed that the F^2 BfR females possess the gametic constitution of BfRX–BfWX. Both of the combinations BfR and BfW have been produced in the normal percentage by a crossing over in the F^1 generation of factors originally coupled in the grandparents.

The F^2 LW males have been similarly produced, their factors L and W being crossovers.

All combinations, therefore, introduced into the F^1 generation have been produced by a rearrangement of the original combinations owing to crossing over.

Table XVII. shows the expected results of such a crossing.

Table XVII.

To Illustrate Experiment X. (Crossovers.)

P^1 BfRX – BfWX = BfR ♀ LWX – o = LW ♂
Gametes BfRX, BfWX LWX, o

F^1 BfRX – LWX = LR ♀ BfRX – o = BfR ♂
BfWX – LWX = LW ♀ BfWX – o = BfW ♂
LR of F^1 × BfW of F^1

F$_1$ Eggs { (1) BfRX, (2) LWX, (3) BfWX, (4) LRX Sperm { BfWX, o

F^2 BfRX – BfWX = BfR ♀ BfRX – o = BfR ♂
LWX – BfWX = LW ♀ LWX – o = LW ♂
BfWX – BfWX = BfW ♀ BfWX – o = BfW ♂
LRX – BfWX = LR ♀ LRX – o = LR ♂

In spite of the conditions of the experiment the combinations introduced into the F^1 generation persist with a strength of linkage approximately equal to that existing among the original combinations.

Table XVIII. gives the actual results of the experiment.

TABLE XVIII.

RESULTS OF EXPERIMENT X.

BfR ♀ × LW ♂ (Both crossovers from F^2 of Table 16).

F^1 LR and LW ♀ ♀, BfR and BfW ♂ ♂

LR ♀ of F^1 × BfW ♂ (from stock bottle)

F^2		♀					♂				
Bottle No.	Transfer.	BfR	LW	BfW	LR		BfR	LW	BfW	LR	
17	a	89	62	1	3		78	64	5	2	
	b	46	39	1	0		41	46	0	1	
	c	33	31	0	2		26	32	3	3	
	d	39	46	2	3		33	32	2	1	
	e	28	28	0	5		23	29	4	3	
	f	2	0	0	0		2	4	0	0	
		237	206	3	13	3	203	207	14	10	6
18	a	32	37	3	3		38	30	3	0	
	b	56	41	3	2		56	48	5	2	
	c	21	22	3	0		21	15	2	0	
	d	31	29	2	3		32	24	5	4	
	e	27	31	0	3		38	28	3	2	
	f	6	5	1	0		4	5	0	0	
		173	165	12	11	6	189	150	18	8	7
19	a	49	57	2	5		67	62	3	1	
	b	30	30	2	3		26	29	2	2	
	c	28	23	2	3		22	18	3	1	
	d	37	43	0	4		38	32	5	1	
	e	16	14	3	0		15	22	1	3	
	f	5	1	0	0		4	4	0	0	
		165	168	9	15	7	172	167	14	8	7
20	a	65	65	0	5		63	59	8	1	
	b	42	31	1	1		27	47	5	2	
	c	34	46	2	1		38	40	1	1	
	d	54	31	6	1		48	29	3	1	
	e	4	3	0	1		9	3	0	1	
		199	176	9	9	5	185	178	17	6	6
21	a	19	38	1	2		37	39	3	0	
	b	31	37	2	2		37	51	4	2	
	c	52	48	4	1		36	38	2	2	
	d	65	53	3	2		60	53	4	5	
	e	19	13	0	1		22	7	1	0	
		186	189	10	8	5	192	188	14	9	6
22		159	134	5	6	4	185	135	9	6	4
23		142	115	4	2	2	135	106	4	5	4
24		16	13	0	0		10	9	0	0	
25		62	35	4	1	5	50	37	1	0	1
26		44	31	2	2	5	30	36	0	5	6
27		64	56	3	2	4	64	52	0	5	4
28		92	89	1	8	5	80	89	3	6	5
29		97	92	4	6	5	100	70	5	10	8
30		48	52	3	0	3	55	62	2	1	3
31		98	104	4	8	6	99	85	6	13	9
Total		1782	1625	73	91	5	1749	1571	107	92	6

On examining the numbers from the individual bottles only the normal fluctuation in the strength of linkage is noticeable.

SUMMARY.

The strength of linkage between the factor for bifid wing and the factor for vermilion eye is approximately the same (viz. 32 units) in the original cross in its reciprocal, and in the F_2 from the crossovers of the original. (See Experiments I., II. and III., Tables II., III., V. and VI.)

The strength of linkage between yellow body color and bifid wing is constant (viz. 5 units) in the original and in the F_2 from the crossovers (See Experiment IV., Tables VII., VIII. and IX.).

A repetition of Experiment IV. showed a linkage value (viz. 6 units) not significantly different from that previously found. The linkage moreover is constant in the two reciprocal crosses of this experiment. (See Experiment V.; Tables X. and XI.)

The strength of linkage between white eyes and bifid wings is preserved in the crossovers (viz. original 8, crossover 9 units), but is different in the reciprocal (viz., 4). (See Experiments VI. and VII.; Tables XII., XIII. and XIV).

A modification of experiments VI. and VII. gave a strength of linkage (viz. 5 and 6), which approaches that of the reciprocal (viz., 4) but not the original linkage value (viz., 8). (See Experiments VIII. and IX.; Tables XVI. and XVIII.)

In all of the experiments of this paper the strength of linkage is apparently not changed by a previous crossing over between the factors in question.

The linkage value given by the females is the same as that given by the males of the same experiment. (See Tables XVI. and XVIII.)

Crossovers appear in the F_2 generation equally frequently among the first flies hatched as among those hatched last. That the factor for yellow body color has an effect on the viability of flies is evidenced from the deficient numbers of yellow flies in Tables VIII., X. and XI. A deficiency also occurs in flies with white eyes as compared with those possessing red eyes. (See Tables XIII., XVI. and XVIII.) A bifid wing factor, however, does not seem to have any such effect; the bifid winged flies comparing favorably with the long winged flies (see Table XVIII.).

UNIVERSITY OF CINCINNATI,
May 6, 1914.

NOTES ON REGRESSION IN A PURE LINE OF PLANT LICE.

H. E. EWING.

In a previous number of the BIOLOGICAL BULLETIN[1] the writer published the results of some selections made within a pure line of plant lice. These were made in an attempt to increase and to decrease the length of the third segments of the antennæ in comparison with that of the fourth segments. The species used was our common European grain aphis, *Aphis avenæ* Fab.; and the results obtained up to that time, which included the first ten generations, appeared to show that selections from extreme variants did not alter the mean as obtained for the line without selection. These results were in accord with the results obtained by other workers in pure lines.

But it also appeared that the mean of the offspring of the variants selected reverted not to the mean of the line, or strain, but that it would swing pendulum-like much beyond this mean only to be brought back to the former side of the mean-of-the-strain base line in the next generation.

Since the publication of this former paper I have tested further this aspect of regression by selecting from opposite extremes in alternating generations. These selections were begun in the ninth filial generation, and continued into the fifteenth, including in all seven generations. I will now give briefly the results of these selections.

An individual with the remarkably high index of 2.82 : 1 (*i. e.*, the third segments of the antennæ were two and eighty-two hundredths times as long as the fourth segments) was selected as the stem progenitor from among the individuals of the F_8 fraternity,—which fraternity has been mentioned in my previous paper. From this individual there were reared five wingless adults which gave a fraternal mean of 1.75 : 1, *i. e.*, .05 below the mean of the strain which was 1.80 : 1. From the F_9 fraternity,

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[1] Vol. XXVI., No. 1, January, 1914.

$F_9$16 was selected for carrying on the strain. It had an index of 1.58 : 1, and gave a fraternity with a mean of 1.82 : 1. From this F_{10} fraternity, F_{10}4 with an index of 2.11 : 1 was chosen. The mean for its offspring was 1.89 : 1. From this F_{11} fraternity, F_{11}4, formula 1.78 : 1, was isolated. It gave the F_{12} fraternity, with a mean of 1.84 : 1. F_{12}6, formula 1.98 : 1, gave the F_{13} fraternity, with a mean of 1.85 : 1. F_{13}4, the next parent selected, had an index of 1.67 : 1, and gave the F_{14} fraternity with a mean of 1.69 : 1. From the F_{14} fraternity the last selection was made. F_{14}1, formula 1.70 : 1, was isolated, and gave the F_{15} fraternity with 1.81 : 1 as a mean.

In each of these selections the individual with either the highest or the lowest antennal index was isolated in order to obtain the next generation of descendants. As has been stated, the index as obtained without selection for the mean of the pure line was 1.80 : 1. Thus it will be noticed that in four of the seven selections made, the offspring of the extreme variant gave a fraternal mean which showed a regression beyond the mean of the strain. In the case of the other three selections the regression of the fraternal mean did not reach the mean of the strain.

In order to observe more fully the effects of selection and the action of regression, I will give here in the order of their ratios the antennal formulæ of the eighteen parents thus far obtained in the first fifteen generations of the pure line[1] together with the indices representing the fraternal means of their offspring. They are as follows:

Indices for Parents.	Indices for Fraternal Mean of Offspring.
1.66	1.88
1.67	1.77
1.67	1.83
1.67	1.69
1.70	1.81
1.77	1.77
1.77	1.71
1.78	1.84
1.79	1.95
1.80	2.01
1.85	1.80
1.86	1.77

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[1] Two of the parents which had antennal indices such as to suggest mutations or abnormalities are omitted.

1.86	1.85
1.88	1.80
1.89	1.93
1 98	1.85
2.08	1.66
2.11	1.89

If we now plot these results by using a scheme similar to that employed to illustrate Galton's law of regression we shall have the following graphical representation (Fig. 1) of the regression as found up to the fifteenth generation in this pure line of plant lice.

Here the heavy line represents the mean-of-the-strain base line, and is placed at 1.80. The lighter parallel lines above and below this base line each represent an increase or decrease in the index ratio for the third and fourth antennal segments of five one-hundredths. Similarly one of the vertical lines represents the mean-of-the-strain base line, and is marked 1.80; and on

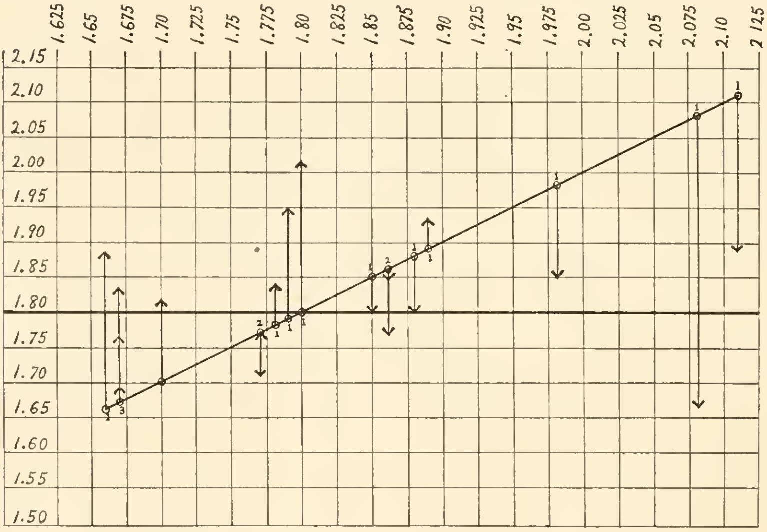

FIG. 1.—Diagram showing regression within a pure line of *Aphis avenæ* Fab. The heavy base line at 1.80 represents the position of the mean of the line, or strain. The small circles represent the positions of the parents according to the index of their third and fourth antennal segments; the small number placed by each circle shows the number of parents with the antennal index indicated by the circle; the arrow-heads represent the positions of the mean of the fraternities obtained from these various parents. The arrows show the amount of regression.

each side of it other parallel lines are laid off at equal distances. Each of these represents a difference of 0.025 in the index ratio for the antennal segments considered.

On this diagram small round circles have been placed representing the position of each of the eighteen parents according to their antennal index-ratio. These naturally form a straight line which is indicated. Now the mean index of the offspring of each parent is represented by an arrow-head, placed immediately above or below, as the case may be, the circle indicating the index of the parent. A line connecting these two points forms the arrow which indicates the actual amount of regression in each instance. In three instances we have more than one stem parent with the same index-ratio, hence in these cases the arrows showing regression are superimposed.

It is observed that in some instances the regression is not to the mean of the strain, and in other instances it is much beyond the mean of the strain; while in two instances there is no regression at all, but a deviation from the mean of the strain even greater than that that existed before in the parent.

In order to compute the average amount of regression we may arrange in parallel series the deviations as shown in the indices of the various parents from that of the mean of the strain, and the deviation shown by the average for the indices of their progenies. This is here done, the mean of the strain being given as zero and the deviation found in the various antennal ratios from this mean being given in hundredths, plus or minus, as the case may be.

Deviations in indices of parents	− 14	− 13	− 13	− 13	− 10	− 3	− 3	− 2	− 1
Average deviation found in offspring	+ 8	− 3	+ 3	− 11	+ 1	− 3	− 9	+ 4	+ 15
Deviations in indices of parents	0	+ 5	+ 6	+ 6	+ 8	+ 9	+ 18	+ 28	+ 31
Average deviation found in offspring	+ 21	0	− 3	+ 5	0	+ 13	+ 5	− 14	+ 9

This relation of the regression can be expressed in the form of fractions by taking in each instance the difference in the deviation of the offspring from that of the parent, for the numerator; and the deviations of the parent itself in each instance as the denominator. These will then be:

+ 22/14, + 10/13, + 16/13, + 2/13, + 11/10, 0, − 6/3, + 6/2, + 16/1, + 5/5, + 9/6, + 1/6, + 8/8, − 4/9, + 13/18, + 42/28, + 22/31.

We may also express this series in the form of decimals, which will be as follows:

1.57, .76, 1.23, .15, 1.10, 0, − 2.00, 3.00, 16.00, 1.00, 1.50, .16, 1.00, − .44, .72, 1.50, .70.

These fractions added together and divided by their number should give us the average amount of regression. If the regression is according to Galton's law the decimal should be 0.333; if according to Johannsen's predictions, that is if the regression is complete, it should be 1.00. The figure which we actually obtain by adding these fractions and dividing by their number is 1.64. In other words, the regression is more than complete, or beyond the mean of the strain. However, it should be noted that the number of individuals included in this computation is too small to permit the results to be conclusive. Yet the results show that regression in a pure line of a parthenogenetic form does not follow Galton's law, also that there appears to be some justification in the contention made in a previous paper of mine, that regression under these conditions is somewhat pendulum-like, swinging beyond the mean of the strain, or line.

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BIOLOGICAL BULLETIN

REGENERATION OF *PLEUROTRICHA* AFTER MEROTOMY WITH REFERENCE ESPECIALLY TO THE NUMBER OF MICRONUCLEI AND THE OCCURRENCE OF UNINUCLEATE CELLS.[1]

J. H. HEWITT.

Lewin,[1] working with *Stylonychia*, has reported a series of experiments in which, after merotomy, he observed an increase in the number of micronuclei in the regenerated merozoa. It was thought to be of interest to repeat Lewin's experiments on another member of the hypotrichus group to determine if there might be any general application of the phenomenon he had observed. Pleurotricha was chosen for the experiments.

Material.

The animals used were secured from a strain, the originator of which was isolated by Dr. George A. Baitsell in the biological laboratory of Yale University. The strain had been adapted to laboratory hay infusion media and was preserved as a stock culture in sterile test-tubes plugged with cotton. Such a culture tube of organisms was given me and from it subcultures were made to other tubes of fresh media and to fresh media in small glass capsules.

The hay infusion used as medium was prepared by placing about 10 grams of field hay in 200 c.c. of tap water in an Erlenmeyer flask and boiling it over a bunsen burner for a few minutes. This medium was made up but once and was used in the proportion of one drop of the infusion to five drops of tap water, this amount of fluid in a capsule serving as the medium for a single animal for twenty-four hours, or till it divided.

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[1] From the Marine Biological Laboratory, Woods Hole, Mass.

METHOD.

Merotomy was performed according to the method of Calkins. The animal selected was drawn up into a fine pointed pipet and placed on a clean glass slide under a Greenough's binocular microscope, eye-pieces 4 and objectives a. The medium was then drawn off till a drop of only sufficient size for the animal to swim in freely was left. With an ophthalmologist's iridectomy knife the animals were cut in parts. The point was ground off the knife and one edge ground to a semi-bellied shape. The blade was plunged into the drop with the animal and the posterior point of its edge allowed to rest on the surface of the slide. As the animal passed from one side to the other of the drop, or around the resting point of the knife edge, successive attempts were made by moving the knife handle up and down to cut the animal as it came directly in line with the edge of the knife.

The frangibility of the cell body afforded one of the first methods of securing fragments of infusoria for study. *Pleurotricha* appears to be very frangible. On one occasion, experiment 48, catching an animal on the surface of the media with a sudden and forcible blast of air from a fine pointed pipet the animal was broken in two. Simply drawing the animal rather forcibly in the pipet was sometimes sufficient to break it in two as in experiment 40. In a few of the experiments the merozoa were secured by drawing the animal selected into a fine pointed pipet and spurting it out forcibly on the side of the capsule.

Animals to be stained were isolated from the capsule with a fine pipet on a clean microscopical slide under a binocular microscope. They were killed and fixed in 5 per cent. glacial acetic acid in saturated mercuric chloride, stained by the Heidenhain iron hematoxylin method, and mounted in xylol balsam.

THE EFFECT OF MEROTOMY ON THE NUMBER OF MICRONUCLEI.

In Tables I. to V., inclusive, are recorded 27 experiments in which the regenerated merozoön was recovered, successfully stained, and mounted. The tables are divided according to the position of the cut. They also state the exact time before or after division, when it had been observed, the length of time after merotomy before the animal was killed, and the number of macronuclei and micronuclei found in the stained merozoön.

In experiments 31, 32, 33 and 57 the animals were killed from 38 minutes to 3 hours and 30 minutes after merotomy. All of these, except experiment 31, were mid-body cuts and it is reasonable to assume that the condition found is normal,—the macronucleus and the micronucleus having not yet divided, or being cells of the uninucleate variety which were also found in control cultures of this animal. In experiment 57 the micronucleus is in mitosis and the cell appears normal.

Table I.

Anterior-end Cuts.

Exp. No.	When Cut.	When Killed.	Number of Macronuclei, Number of Micronuclei.
45	—	9 hrs. 53 min. after cut.	2 macronuclei, 2 micronuclei
105	Before division	27′ 25″ after cut.	2 macronuclei, 2 micronuclei

In experiment 22, also a mid-body cut, 1 hour and 10 minutes after operation, there are two micronuclei in mitosis. The nucleus is single, swollen and enlarged, as if ready to divide. The most plausible explanation of the condition found here is that the cell originally had two macronuclei and three micronuclei, as was found in certain animals taken from culture. When merotomy was performed the animal was separated in two merozoa, one of which had a single macronucleus and a single micronucleus; the other merozoön which was saved and which regenerated had a single macronucleus and two micronuclei.

Table II.

Posterior-end Cuts.

Exp. No.	When Cut.	When Killed.	Number of Macronuclei, Number of Micronuclei.
4	1 hr. 7 min. after division.	3 hrs. 18 min. after cut	2 macronuclei, 2 micronuclei.
15	Before division	6 min. after cut	2 " 2 "
27	"	30 " " " .	2 " 2 "
33	"	38 " " "	1 " 1 "
34	"	1 hr. 10 min. after cut	2 " 9 "
39	"	8 hrs. 27 " " "	2 " 2 "
49	"	18 hrs. 7 " " "	2 " 2 "
106	"	27 hrs. 15 " " "	2 " 2 "
107	"	26 hrs. 55 " " "	2 " 2 "
109	"	22 hrs. 36 " " "	2 " 2 "

In experiment 34, a posterior-end cut, 1 hour and 10 minutes after operation, we have an abnormal and an interesting animal. The cell is pointed at both ends and broad in the middle, measuring 112 x 45 micra. The anterior macronucleus is situated nearer the anterior end than normally. It is round in shape, measuring 5 micra in diameter. Its chromatin is homogeneous, deeply stained, showing no vesicles or granules. The posterior macronucleus is likewise situated nearer the anterior end than normally. It measures 7 x 5 micra and its chromatin is of the same character as that of the anterior macronucleus. There is one micronucleus adjacent to each macronucleus and seven other micronuclei scattered irregularly throughout the anterior portion of the cell. They are all of about the same size, 2 micra in diameter, deeply stained and homogeneous. The cytoplasm

TABLE III.

MID-BODY CUTS.

Exp. No.	When Cut.	When Killed.	Number of Macronuclei, Number of Micronuclei.
7	1 hr. 17 min. after division.	3 hrs. 15 min. after cut.	2 macronuclei, 2 micronuclei.
22	Before division	1 hr. 10 min. " "	1 macronucleus, 2 "
25	" "	2 hrs. 25 " " "	2 macronuclei, 2 "
31	" "	2 " 30 " " "	1 macronucleus, 1 micronucleus.
32	" "	3 " 30 " " "	1 " 1 "
35	" "	11 " 40 " " "	2 macronuclei, 2 micronuclei.
47	6 hrs. 8 min. after division.	14 " 47 " " "	2 " 2 "
51	6 hrs. 3 min. after division.	13 " 3 " " "	2 " 2 "
57	———	1 hr. 35 " " "	1 macronucleus, 1 micronucleus. (dividing)
88	Before division.	14 hrs. 3 " " "	4 macronuclei, 4 micronuclei.
93	" "	41 " 30 " " "	2 " 2 "
95	" "	45 " 21 " " "	2 " 2 "

TABLE IV.

ANTERIOR- AND POSTERIOR-END CUTS.

Exp. No.	When Cut.	When Killed.	Number of Macronuclei, Number of Micronuclei.
99	Before division.	43 hrs. 47 min. after cut.	2 macronuclei, 2 micronuclei.

is finely granular. The changes here appear more like those of a degenerative than those of a physiological process and may have been present at the time the animal was operated upon.

In the twenty other experiments there were two macronuclei and two micronuclei, the number normally and most constantly found in this strain of animals.

TABLE V.

LONGITUDINAL CUTS.

Exp. No.	When Cut.	When Killed.	Number of Macronuclei, Number of Micronuclei.
86	Before division.	20 hrs. 20 min. after cut.	(dividing) 4 macronuclei, 4 micronuclei.
110	" "	25 hrs. 50 min. after cut.	2 macronuclei, 2 micronuclei.

In Table VI. are recorded the observations made on twelve animals, descendants of merozoa and selected from various generations from the second to the thirty-fifth. In all of these animals there were two macronuclei and two micronuclei.

TABLE VI.

Exp. No.	Cut.	Generation after Cutting.	Number of Macronuclei, Number of Micronuclei.
16	Anterior.	35th	2 macronuclei, 2 micronuclei.
21	Posterior.	30th	2 " 2 "
25	Mid-body.	24th	2 " 2 "
37	Anterior.	10th	2 " 2 "
53	"	2d	2 " 2 "
61	Mid-body.	4th	2 " 2 "
64	Posterior.	2d	2 " 2 "
64	"	3d	2 " 2 "
70	"	2d	2 " 2 "
70	"	3d	2 " 2 "
85	Anterior.	2d	2 " 2 "
106	Posterior.	2d	2 " 2 "

Thus, the evidence furnished by these experiments is that merotomy does not produce any change in the normal number of macronuclei and micronuclei, either in the merozoön or in its descendants as far as the thirty-fifth generation. The occurrence of cells with more micronuclei than macronuclei are more readily explained as having been mechanically produced by the operation itself or as the manifestation of an abnormal cell process that may have existed before merotomy was performed, as such cells are found in normal laboratory cultures of this animal.

The Occurrence of Animals Containing but One Macronucleus.

On four occasions animals from the stock cultures and from the cultures of the control strains when killed and stained were found to possess but one macronucleus and sometimes one and sometimes two micronuclei. These animals were all large slow swimmers or crawlers, and were selected because experience had taught that these were the animals that showed early stages of division, which were being sought at that time.

Among the descendants of merozoön c, experiment 24, a uninucleate animal with two micronuclei was found. The original merozoön was from an animal cut during division. From this it was thought that perhaps injury to the cell during division might be the cause of this occurrence, but out of a large number of animals out of this strain and other strains derived from animals cut during division no other animals with a single macronucleus were found.

In experiment 76 a large slow crawler, evidently near division, was cut anteriorly at 12:25 P.M., August 9. The anterior merozoön disintegrated immediately, the posterior merozoön increased its activity. It was isolated into a clean glass capsule with five drops of tap water and one drop of hay infusion. On August 10, 10:15 A.M., there was found in the capsule three animals, two small and of the same size and a single large animal. The interpretation made of this was that the merozoön had regenerated, divided once into two individuals and that one of the latter had again divided while the other was now approaching division. On killing and staining all three of these animals it was found that each of the small animals had two macronuclei and two micronuclei, but the large animal had only one macronucleus and two micronuclei. The macronucleus appeared about to divide.

Discussion.

Since uninucleate forms occur both among the stock cultures, the control strains and among the merozoa, a conservative inference must be that they are normal variations of this animal, possibly brought about by its being adapted to laboratory media. The relation between the two may be somewhat analogous to that which Calkins[2] has found to exist between the so-called *Paramecium caudatum* and *Paramecium aurelia*, except that there it is a variation in the number of micronuclei; here, a variation in the number of macronuclei. It appears possible that by proper selection a strain of Pleurotricha may be obtained in which all of the animals, for a period, may show only one macronucleus.

From the experiments it appears fairly conclusive that merotomy generally has no effect on the normal number of micronuclei. Animals with less macronuclei than the normal may be found both in laboratory cultures of this animal and among the descendants of merozoa. Animals with more than the normal number of micronuclei may occur in laboratory cultures. The single instance in which there was found among the merozoa an animal with two abnormal macronuclei and several micronuclei is undoubtedly that of a degenerative or a necrobiotic cell. This is somewhat suggestive that multiple micronuclei may in general be one of the manifestations of a degenerative or a necrobiotic process in the cell.

It is to be noted that these observations are not in accord with Lewin's findings for *Stylonychia*. It appears from his paper that he had some difficulty in getting his animals to survive in the media used and another possible explanation for his observations is that they were more the results of necrobiosis and degeneration in the culture strains than the effect of merotomy.

Conclusions.

1. Merotomy has no effect other than the effect that may be mechanically produced by the operation itself on the number of micronuclei of *Pleurotricha*.

2. Animals with more than the normal number of micronuclei and less than the normal number of macronuclei may occur in laboratory strains of this animal as well as among merozoa.

Acknowledgments.

I am indebted to Dr. G. N. Calkins for suggesting this study to me and for assistance, also to Dr. George A. Baitsell for the

cultures of *Pleurotricha* with which the work was performed. To each of these men I desire to express my sincere appreciation.

REFERENCES.

1. **Lewin.**
'11 Royal Soc. Bulletin, Vol. 84, p. 333.
2. **Calkins.**
Protozoology, New York and Philadelphia, p. 112.

THE REACTIONS OF CRAYFISHES TO GRADIENTS OF DISSOLVED CARBON DIOXIDE AND ACETIC AND HYDROCHLORIC ACIDS.[1]

EDWIN B. POWERS.

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I. INTRODUCTION.

The following experiments were undertaken to determine the reactions of crayfishes to gradients of acids, and, if possible, to determine the relation of the distribution of carbon dioxide contained in water to the natural distribution of the crayfishes. It was also hoped that something might be added to the present knowledge of the physiology of rapid modification of animals in gradients. The experiments were conducted between January 13 and April 27, and between June 16 and July 31, 1913. The first set will be designated as low-temperature experiments and the second set as high-temperature experiments.

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[1] From the Hull Zoological Laboratory, University of Chicago.

II. Material and Methods.

1. *Apparatus and Method of Experimentation.*

In the study of reactions of the crayfishes in gradients of carbon dioxide and acetic and hydrochloric acids, the method and apparatus were devised by Shelford and Allee ('13) for the study of reactions of fishes to gases or solids in solution. The apparatus, a full description of which is given in Shelford and Allee ('13) "The Reactions of Fishes to Gradients of Dissolved Atmospheric Gases" (pp. 225–229), consists of two tanks each 120 cm. long by 20.5 cm. wide by 14 cm. deep with outlets at the center of both sides, near the top. These outlets are guarded by a screen-bottomed tube which extends across the tank. The tanks are placed side by side in an aquarium, beneath a hood under identical and symmetrically surrounding conditions. Tap water was introduced at both ends through perforated tees behind screens. In the experiments with carbon dioxide, the flows were 600 c.c. per minute. At one end carbon dioxide was introduced into the inlet so that a gradient was produced lengthwise of the tank between the tap water and the water high in carbon dioxide. This was shown by titrations made of samples taken from different portions of the tank. In the acetic and hydrochloric acid experiments the conditions were the same, except for the flow at the tap water end and corresponding end of the control, which was 1,200 c.c. per minute, thus producing a sharper gradient. This flow was also used in all high temperature carbon dioxide experiments. The acetic and hydrochloric acids were introduced by means of a separate tee.

After everything was made ready for the keeping of records, the crayfishes were dropped into the center of the tank and were observed through a slit in the hood, and the back and forth movements recorded in the form of a graph on paper especially prepared for this purpose. Records were also kept of specific reactions observed in any individual. These records were made between the spaces set aside for the graphs. The previous history of the animals and the gradient used with a description of conditions of experimentation were entered at the top of the page. After the crayfishes were dropped into the middle of the tank they were disturbed as little as possible. At the end of each experiment a titration of samples of water taken from the two ends and center was made with sodium carbonate and recorded.

2. *Stock.*

The crayfishes were of the species *Cambarus propinquus* Gir., *C. virilis* Hag., *C. diogenes* Gir. and *C. immunis* Hag. They were all of medium size with the exception of a few specimens of *C. virilis* and *C. diogenes* which were above medium size. *C. propinquus* and *C. virilis* were all taken at New Lenox, Ill., from the pools just above and just below the rapids and from among the rocks of the rapids of Hickory Creek. The first stock was obtained December 25, 1912, and was kept in the laboratory in a large pan with the bottom covered with sand, gravel and some vegetation. The water was changed six times per week. When brought in a few died during the first three to five days, due possibly to the sudden change of temperature, but after this there was little mortality. Stock obtained November 15, 1912, and January 25, 1913, which were kept in an aquarium, suffered the same experience when transferred to the pan. Stock obtained March 22 and 29 and June 21, 1913, was kept in an aquarium in which was a strong flow of water to lower the temperature to more nearly that of experimentation. These suffered no great mortality. *C. immunis* was obtained from sloughs, mostly from the bottom, and a few from burrows, and *C. diogenes* from burrows only, along the banks of the same sloughs near Clark Junction, Ind., June 20 and 24 and July 9 and 26. These were all kept in large glass jars into which fresh water was flowing. There was no great mortality experienced by these stocks.

3. *Habitat.*

C. propinquus is essentially a quiet water stream form. It inhabits the pools with more or less muddy bottom (Williams, '01). They hide under rocks or rest quietly on the bottom.

Sometimes they lie concealed in short burrows along the banks (Harris, '03). *C. virilis* is found to be absent from the muddier and shallower portions of the streams but is sometimes taken in ponds with *C. immunis* (Harris, '01), but is more often found in running streams, among the rocks of the more rapid portions (Harris, '03). They do not burrow except when the ponds begin to dry or winter approaches. Garman ('89) took this species from wells, Wilson's cave and streams. *C. diogenes* is a burrowing species and often makes mud chimneys, often burrowing in damp ground some distances from the open water, which it seldom enters except during the breeding season (Pearse, '10). *C. immunis* is a mud-loving species and is found mostly in small pools, though it sometimes occurs in brooks and rivers (Pearse, *l. c.*) (Harris, '03).

4. *Senses.*

The fact that crayfishes are sensitive to chemicals has been shown by several authors. Putnam ('75) noted that *C. pellucidus*, the blind crayfish, will hunt food when it is thrown into the water. Holmes and Homuth ('10) found that the whole body is more or less sensitive to olfactory stimuli, and that the antennæ, mouth parts and tips of the chelipeds are sensitive in the order named. Nagel ('94) observed the chemical sense in *Astacus*. Wright ('84), by a study of the antennules of *C. propinquus*, found that five of the eighteen segments, *i. e.*, eleven to fifteen inclusive, bear eight of the so-called olfactory organs and the distal nine fewer. Bell ('06) found that the crayfishes with which he worked reacted positively to meat juice and negatively to lavender water, acids and salts, and concluded that they are sensitive over the entire body but more in the anterior appendages than in other parts. Chidester ('12) found when meat was thrown into the water that crayfishes would approach and seize fresh meat quicker than they would meat dried in the air.

III. The Sensing of Carbon Dioxide and Acetic and Hydrochloric Acids.

The crayfishes sensed the carbon dioxide and acetic and hydrochloric acids when passing into the high acid concentration end of the experiment tank. This was indicated by certain specific reactions which were made as the crayfishes entered the higher concentration. Such reactions were waving the antennæ, moving the appendages, backing and walking with the legs extended. The waving and moving of the appendages were especially noted in the reactions of *propinquus*, *virilis* and *diogenes*, in the carbon dioxide experiments in which the concentrations of carbon dioxide in the two ends were 40 to 60 and 80 to 100 c.c. per liter respectively. The animal would also crouch down in the corner of the tank. This tendency to wave the antennæ and move the appendages was present in *virilis* in the acetic acid also. The backing reaction was not so common in the carbon dioxide experiments but was marked in the hydrochloric and acetic acid experiments.

IV. The Effect of Carbon Dioxide and Acetic and Hydrochloric Acids.

1. *The Effect on Reflex of the Crayfishes.*

The crayfishes not only detected the presence of the acids but were intoxicated or anæsthetized by them in the low temperature experiments, possibly due to the less regular movements of the animal. This is shown in graphs (Chart I.) by the longer periods of time required to cross the tank in the experiment than that required in the control. The effect was greatest in the experiments with high concentrations. The first effect of the carbon dioxide was to interfere with the correlation of movements and to cause the animal to carry the body high with legs extended. Progressively locomotion became slower and slower until it ceased, but the appendages were still moved one after the other. The crayfish would finally fall upon its back and continue to move its appendages for a short time, after which it would remain motionless as if dead. It would recover rapidly if placed in fresh water and after a short time move about normally. The acetic acid produced this same effect upon *virilis* but *propinquus*, *diogenes* and *immunis* were either not intoxicated by the acetic and hydrochloric acids or acted rapidly enough to get out of the high concentration before being greatly affected, (see Charts I. and II.). The movements of *virilis* were always more or less irregular. One of the individuals in experiment 21 was completely anæsthetized but recovered in eight minutes when placed in fresh water. At first it remained motionless in the fresh water but later revived and seemingly became normal.

2. *Anæsthesia and Death.*

An experiment was performed to determine the relative resistance of the four species of crayfishes to high concentration of carbon dioxide. Though the experiment was terminated before the crayfishes had all been killed, through an accident to the apparatus, it had been carried sufficiently far to determine the susceptibility of the four species to the carbon dioxide solution. The apparatus consisted of large glass bottles (Wells, '13) through which water containing from 6.92 to 7 c.c. of oxygen per liter and varying amounts of carbon was flowing. The amount of the gases were determined by titrations of samples of water collected from the over-flow. The temperature was 21.5° to 23° C.

It was found that in all cases the smaller individuals of a species died first. This was probably due to the greater proportion of surface to mass in the smaller specimens than in the larger ones, rather than a difference of susceptibility of the smaller specimens to the carbon dioxide solution. These are the same general results obtained by Wells (Wells, *l. c.*) with fishes.

All remained active in 50 c.c. carbon dioxide per liter. *Virilis* was anæsthetized by solutions of 120 to 145 c.c. per liter. *Propinquus* was not overcome but showed that they were effected, while *diogenes* and *immunis* showed the effect to a less extent. *Propinquus* was not anæsthetized as early as *virilis* but the time of death approached that of *virilis*, the small specimens of *propinquus* having died before the large *virilis*, but a medium sized *propinquus* survived all the specimens of *virilis*. *Diogenes* and *immunis* were much less susceptible to the carbon dioxide than either *propinquus* or *virilis*. All specimens of both *propinquus* and *virilis* died before the medium-sized specimen of *diogenes*. The specimens of *diogenes* and *immunis* died in the following order: One medium-sized *diogenes*, one *immunis*, one *diogenes*, one *immunis*, two *diogenes* and two of *immunis*. There were one *immunis* and one large *diogenes* alive when the experiment terminated. All the specimens of *immunis* were smaller in size than the smallest specimen of *diogenes*. From this data it seems the four species are susceptible to high concentrations of carbon dioxide in the following order: *virilis*, *propinquus*, *diogenes* and *immunis*.

V. The Reaction and Modification in Gradients.

The reaction of crayfishes in gradients is shown in Tables I. and II. Table I. for carbon dioxide, II. for acetic and hydrochloric acids, are arranged in order of sharpness of the gradient, *i. e.*, the difference between the concentrations of acid at the two ends. The four species are grouped separately in each table. Reaction is shown by the time preference for one end or the other, by turnings and by crossings of the center. There is also tabulated the modification of behavior by turnings accompanied by backing. The backing reaction is not indicated in the ratings (Table III.), which is the numerical expression of avoidance of ends when turnings and time spent in the halves of the tank are considered. The ratio of the increased concentration of the acid of the high end over that of the low end, or Weber's ratio, is tabulated for comparison with the difference of concentration of the acid at the two ends.

The crayfishes, when passing into the high concentration end of the tank, gave certain definite avoiding reactions, when not too much affected by the presence of the acid. Of these the reactions recordable in graphs are (1) turning upon encountering gradient, either the first time or after one or more invasions and (2) reactions which cannot be recorded graphically are turning accompanied by backing and crawling on the screen, out of the water, or attempting to crawl on the sides of the tank. Recordable reactions were in the most cases rhythmic and represented a rapid modification of behavior. Reactions of the first class are grouped in one column of the table, but where backing accompanied turning separate mention is made of this fact. Of the reactions that could not be graphically recorded, that of crawling on the screen is least definite as an avoiding reaction since it was noted in the controls also, though less than in the experiments. This reaction is probably due to thigmotactic

TABLE I.

Showing reaction of crayfishes in a gradient of carbon dioxide. In all experiments and controls three animals were tested simultaneously except in the 20-minute experiments and controls in which case each of the three individuals were tested separately, 20 minutes each. H indicates high carbon dioxide and L low carbon dioxide. Corresponding ends of control tank are designated by the same letters. Typical experiments, only, are given in table. Totals and per cent. are totals for all experiments and controls of each species of crayfishes in each gradient. The experiments of each species are grouped and arranged in order of sharpness of gradient between the two ends of tank. For gradient see Table III.

Experiment No.	*Cambarus* Species.	Carbon Dioxide in c.c. per L.		Per Cent. of Time Spent in Halves of Experimental and Control Tanks.				Number of Times Crossed Center.		Number of Times Turned in Gradient from High and Low Ends.				Number of Turnings Accompanied by Backings.				Temperature, Centigrade.	Time of Experiment.
				Expt.		Cont.				Expt.		Cont.		Expt.		Cont.			
		L	H	H	L	H	L	Expt.	Cont.	H	L	H	L	H	L	H	L		
2	*Propinquus*. .	3.5	20.	27.9	72.1	51.	49.	23	40	17	4	3	2	5	0	0	1	6.	60
26	"	3.6	23.	21.3	78.7	37.1	62.9	48	36	31	7	7	12	0	0	0	0	17.5	40
5	"	47.4	94.	5.6	94.4	70.6	29.4	7	41	12	1	1	5	4	0	0	0	3.75	60
	Totals.							157	237	124	57	32	40	20	3	3	1		
	Per cent.									71	29	44.5	55.5						
24	*Virilis*.	1.5	9 5	41.7	58.3			39		30	23			2	0			19.	40
27	"	2.9	46.5	11.1	88.9	24.1	75.9	40	18	40	21	14	20	1	0	0	0	17.5	40
	Totals.							108	85	78	54	27	24	4	0	0	0		
	Per cent.									59	41	53	47						
44	*Diogenes*.	2.3	18.9	33.3	66.7	35.8	64.2	18	21	7	5	5	3	0	0	0	0	20.5	20
28	"	56.5	179.4	26.	74.	58.8	41.2	20	32	18	11	4	3	8	2	0	1	17.5	20
	Totals.							58	85	69	45	39	29	15	6	0	1		
	Per cent.									60	40	57.3	42.7						
45	*Immunis*. . . .	2.3	9.4	33.7	66.3	48.3	51.7	52	38	13	5	7	8	0	0	0	0	21.	20
46	"	2.7	24.5	48.5	51.5	54.6	46.4	37	37	13	11	1	2	1	2	0	0	20.5	20
	Totals.							139	65	49	33	8	10	2	3	0	0		
	Per cent.									59.8	40.2	44.5	55.5						

response as well as an avoidance of the acids. The tendencies to crawl out of the water and upon the side of the tank are more clearly acid avoiding reactions. In the controls there were no attempts to crawl on the sides of the tank and few animals showed an inclination to crawl out of the water.

1. *Carbon Dioxide Gradient.*

(*a*) *C. propinquus*.—The crayfishes, upon invading the high carbon dioxide end, showed a tendency to turn before reaching the screen, or they would back a short distance and then turn and pass to the lower carbon dioxide end. Of all the individuals tried 55 per cent. turned back on the first encounter of the gradient and 14.8 per cent. of these turnings were accompanied by backings (Table I.). There was a greater number of turnings from the high concentration end than from the low, there being a total of 124 from the high end to 51 from the low. The fact that turning from the high end is an avoiding reaction is emphasized by 16 per cent. of the turnings being accompanied by backing. There were fewer crossings of the center in the experiments than in the controls, in the latter there being a tendency to travel the entire length of the tank. In some cases the reduction of crossings of the center represents the extent of anæsthetization of the animals.

In the experiments with low concentrations (low 3.5 and high 20 c.c. of carbon dioxide per liter) and low temperature the turnings were rhythmical (Chart I., Expt. 2). Long invasions of the high concentration end were followed by shorter invasions and periods of rest at the low concentration end. These were then followed by very short invasions and very long periods of almost complete rest in the low concentration end. Later there would be a second similar period of invasion and rest. Three such periods are shown in the graph. Thus there is a period of increased sensitiveness or a period of increased reaction to the same incoming sensation, after invasion of the high concentration end. In either case there is a rapid modification of behavior.

With higher concentrations (24–47 c.c. per L., low end and 58–94 c.c. per L. high end), if the crayfishes were not too greatly overcome, there was greater rapidity of reaction and a more

TABLE II.

Showing reaction of crayfishes in gradients of acetic and hydrochloric acids. For further explanation see Table I.

Experiment No.	*Cambarus* Species.	Acetic Acid in g. per L.		Per Cent. of Time Spent in Halves of Experimental and Control Tanks.				Number of Times Crossed Center.		Number of Times Turned in Gradient from High and Low Ends.				Number of Turnings Accompanied by Backings.				Temperature, Centigrade.	Time of Experiment.
				Expt.		Cont.		Expt.	Cont.	Expt.		Cont.		Expt.		Cont.			
		L	H	H	L	H	L			H	L	H	L	H	L	H	L		
14	*Propinquus*..	.24	1.39	3.5	96.5	54.9	45.1	14	22	15	3	11	13	7	0	0	0	4.25	60
13	" ..	2.41	3.66		100.	61.3	38.7	0	16	4	0	6	6	2	0	0	0	4.25	60
	Totals......							45	93	81	15	33	34	33	0	1	0		
	Per cent.....									84.5	15.5	49	51						
21	*Virilis*......	1.21	1.67	41.	59.	45.3	54.7	11	13	17	7	11	5	9	1	1	0	6.25	40
30	"	.266	3.926	3.2	96.8	28.2	71.8	11	15	22	6	6	7	7	0	0	2	16.5	40
35	"	2.88	28.99	23.2	76.8	53.8	46.2	11	24	13	2	6	1	11	1	2	0	17.	20
	Totals......							101	139	113	46	51	40	52	7	3	5		
	Per cent.....									71	29	36	44						
31	*Diogenes*.....	.086	.312	10.	90.	63.9	36.1	21	27	15	2	11	5	3	0	1	1	16.5	20
33	"	.014	.354	41.1	58.9	92.	8.	26	10	19	10	18	2	5	3	4	0	16.5	20
	Totals......							93	117	51	29	43	24	14	10	5	2		
	Per cent.....									64	36	64	36						
37	*Immunis*....	.078	.429	29.4	70.6	53.8	46.2	21	45	24	8	11	9	7	2	0	0	18.	20
34	"	.429	1.437	16.7	83.3	54.1	45.9	6	3	5	5	1	3	2	0	0	0	16.5	20
	Totals......							33	52	34	15	14	16	10	3	0	1		
	Per cent.....									67.4	32.6	46.7	53.3						

TABLE II.—*Continued.*

Experiment No.	*Cambarus* Species.	Hydrochloric Acid in g. per L.		Per Cent. of Time Spent in Halves of Experimental and Control Tanks.				Number of Times Crossed Center.		Number of Times Turned in Gradient from High and Low Ends.				Number of Turnings Accompanied by Backings.				Temperature, Centigrade.	Time of Experiment.
				Expt.		Cont.				Expt.		Cont.		Expt.		Cont.			
		L	H	H	L	H	L	Expt.	Cont.	H	L	H	L	H	L	H	L		
39	*Propinquus*. .	.0061	.0157	9.4	90.4	60.1	39.9	29	30	27	6	1	2	4	0	0	2	20.5	20
47	" . .	.0275	.0932	4.7	95.3	49.6	50.4	28	63	39	2	0	1	3	0	0	0	21.	20
	Totals.							57	93	66	8	1	3	7	0	0	3		
	Per cent.									89.2	10.8	25	75						
41	*Virilis*.	.0075	.067	12.4	87.6	49.3	50.7	11	32	24	8	2	6	14	2	0	2	20.5	20
	Per cent.									75	25	25	75						
42	*Diogenes*.	.0023	.0078	30.5	69.5			39		7	5			2	0			21.	20
43	"	.0053	.0702	13.8	86.2	39.3	62.7	18	49	14	6	11	13	2	0	0	3	21.	20
	Totals.							57	49	21	11	11	13	4	0	0	3		
	Per cent.									66.7	33.3								
40	*Communis*. . .	.0014	.0304	31.5	68.5	49.3	50.7	23	42	18	7	10	8	2	1	0	1	20.5	20
	Per cent.									72	28	44.5	55.5						

Table III.

Showing the vigor of reaction or rating of the crayfishes in gradients of carbon dioxide and acetic and hydrochloric acids. The rating is obtained by subtracting the percentages given for the time preference for the two ends and subtracting the percentage of turnings from the two ends and adding the two remainders (which are considered of different signs since turnings are from opposite end to end preferred) and dividing by two. The table also shows difference in concentration between the two ends and the ratio of increase of gradient at high end over low end or Weber's ratio.

Experiment No.	*Cambarus* Species.	Grams per L. of Acid or c.c. per L. CO_2.		Difference in g. or c.c. per L. between Two Ends of Tank.	Weber's Ratio.	Rating.		
		L	H			Expt.	Cont.	
22	*Propinquus*	1.5	11.8	10.3	6.8	7.		
2	"	3.5	20.	16.5	4.7	53.	9.	
26	"	3.6	23.	19.4	5.4	60.	26.	
8	"	120.	149.	29.	.24	32.	59.	
3	"	24.	58.	34.	1.4	40.	23.	Carbon dioxide gradient.
5	"	47.4	94.	46.6	.98	86.0	53.	
4	"	43.	90.	47.	1.09	+5.5	20.	
6	"	32.	82.	50.	1.5	32.	59.	
29	"	37.1	154.7	117.6	4.3	42.	32.	
24	*Virilis*....	1.5	9.5	8.	6.3	24.		Ratio of increase of gradient at high end over low end or Weber's ratio.
10	"	18.	54.	36.	2.	+43.	46.	
11	"	62.5	101.	38.5	.26	2.	54.	
27	"	2.9	46.5	43.6	15.	54.	17.	
9	"	86.	172.	86.	1.	38.	3.	
41	*Diogenes*...	2.3	18.9	16.6	7.6	24.	27.	
25	"	3.3	35.9	32.6	9.9	13.	1.5	
28	"	56.5	179.4	122.9	2.1	36.	1.	
45	*Immunis*. .	2.3	9.4	7.1	3.	38.	2.	
23	"	1.3	9.6	8.3	6.4	+ 4.		
46	"	2.7	24.5	21.8	7.7	4.	21.	
17	*Propinquus*	.045	.183	.138	3.2	79.	25.	
16	"	.045	.3	.255	5.7	83.	9.	
15	"	.24	.69	.45	1.9	65.	7.	
14	"	.24	1.39	1.15	5.2	79.	8.	
13	"	2.41	3.66	1.25	.48	100.	11.	
18	*Virilis*....	.018	.114	.096	5.1	53.	11.6	
19	"	.018	.22	.202	11.3	52.	25.	Acetic acid gradient.
21	"	1.21	1.67	.46	.3	39.	8.6	
20	"	.122	.8	.67	5.5	15.	10.	
30	"	.266	3.926	3.66	13.7	75.	21.	
35	"	2.88	28.99	26.11	9.	63.	16.	
31	*Diogenes*...	.086	.312	.226	18.5	78.	1.4	
33	"	.014	.354	.3406	23.8	24.	92.	
38	"	1.027	2.938	1.911	18.	.6	2.2	
36	"	4.97	23.33	18.36	3.6	8.	4.7	
32	*Immunis*. .	.0109	.1105	.0996	9.1	38.	20.	
37	"	.078	.429	.351	4.5	45.	1.2	
34	"	.429	1.437	1.008	2.3	33.	29.	
39	*Propinquus*	.0061	.0157	.0096	1.6	72.	26.	
47	"	.0275	.0932	.0657	2.3	90.	45.	
41	*Virilis*....	.0075	.067	.0595	7.9	63.	12.	
42	*Diogenes*...	.0023	.0078	.0055	2.4	28.		Hydrochloric acid gradient.
43	"	.0053	.0702	.0649	12.2	56.	7.	
40	*Immunis*. .	.0014	.0304	.029	20.2	40.	6.	

rapid modification of behavior (Chart I., Expt. 5). Periodicity is still present but is marked by shorter invasions of the high end. This periodicity is better shown by individuals than by groups. The graph of experiment 5, Chart I., shows that there was a complete cessation of invasions of the high end after 48 minutes and the crayfishes were still resting in the low end after 80 minutes. Numerically expressed, 70 per cent. of all individuals tried showed modification.

(*b*) *C. virilis.*—*Virilis* oriented less definitely to the carbon dioxide gradient than *propinquus.* (Compare ratings, Table III.) The lack of orientation is shown by the percentage of turnings from the two ends and the time preference for one end or the other. This staggering is possibly due to the somewhat more concentrated solutions used, but may be explained as has been suggested by Shelford and Allee ('13) for swift water fishes. That is swift water fishes which encounter very little carbon dioxide may react less definitely to it than fishes which live more often in the presence of carbon dioxide. *Virilis* showed a tendency to crawl out of the water.

(*c*) *C. diogenes.*—*Diogenes* reacted less intensely to both the lower and higher concentrations of carbon dioxide in gradients than either *propinquus* or *virilis.* In experiment 44 with 18.9 c.c. carbon dioxide per liter at the high end there was no backing accompanying the turnings, while in experiment 28, with 179.4 c.c. carbon dioxide per liter at the high end, there were 44 per cent. of the turnings accompanied by backing and one of the turnings on first encounter was accompanied by backing. *Diogenes* also showed a marked tendency in the high concentration carbon dioxide gradient to move forward and then stop after which it would move forward again.

(*d*) *C. immunis.*—All experiments with *immunis* were with concentrations not running above 25 c.c. carbon dioxide per liter at the high concentration end. The avoiding reactions with the exception of experiment 45 were very low. There was a slight positive reaction in experiment 23 as is shown in Table III. There were two turnings from the high concentration end accompanied by backing in experiments 23 and 46 while at the same time there were 3 from the low end.

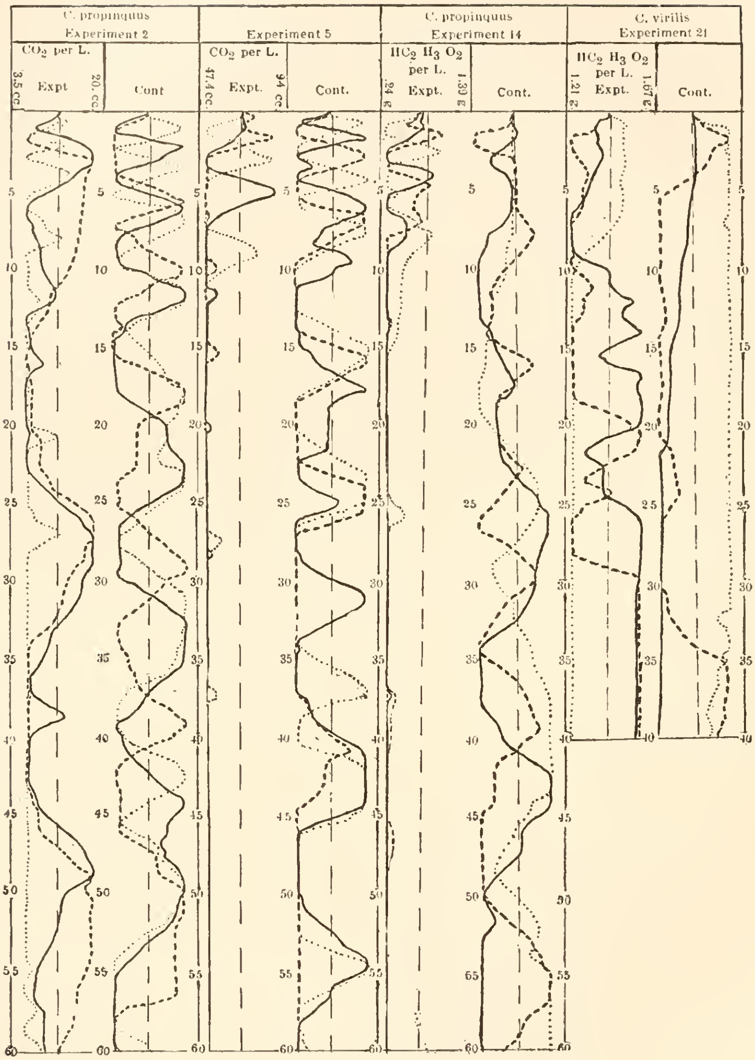

CHART I. Showing reaction of the crayfishes of gradients to carbon dioxide and acetic acid. The horizontal distance between the scales represents the length of the tank. The vertical scale represents time in minutes. Different tracings represent individual animals. Three animals were tested simultaneously.

2. *Acetic Acid Gradient.*

(*a*) *C. propinquus.*—The avoidance of the acetic acid in gradient was sharp and definite (see Chart I., Expt. 14). Orientation was also definite. There seemed to be an acceleration of all the reactions shown in the carbon dioxide experiments. Out of fifteen trials fourteen (93.3 per cent.) showed turning at first encounter of the high concentration, and of the fourteen, four started toward the high end without first having invaded the low or even a portion of it; 50 per cent. of the turnings were accompanied by backing. Only two individuals reached the screen in the acetic acid end. Of these two one crossed after handling; the other occurred in the lowest concentration of acetic acid used. There was, after a certain period of time, a cessation of invasions of the high acid concentration end; this period varied inversely as the total concentration of the acid. Experiment 14 varies slightly from this rule. There was also periodicity of invasions of the high concentration end (see Chart I., Expt. 14) as described in the carbon dioxide experiments.

(*b*) *C. virilis*.—These experiments can be divided into three groups, a low concentration, a high and a very high, *i. e.*, experiments 18 and 19 with a concentration of .0185 g. of acetic acid per liter at the low end and .1145 to .228 g. per liter at the high end; experiments 20 and 21 with .122 to 1.21 and .8 to 1.67 g.; and experiment 35 with 2.88 and 28.99 g. per liter of acetic acid in the low and high ends respectively.

In the low concentration experiments at low temperature there was a more definite orientation and a greater time preference for the low end. This is shown by the ratings (see Table III.). There was also a more or less periodicity of invasions of the high end with a complete cessation of invasions in experiment 19 after 45 minutes.

In the high concentration experiments at low temperature there was less orientation except in turning at the first invasion of the high end. There was but one individual (Expt. 20) that showed periodicity of invasions of the high end. The lack of orientation is shown by graph Expt. 21, Chart I. There was an increased tendency to crawl on the sides of the tank and out of the water. Thus there was a falling off of orientation and a substitution of crawling out of the water.

In experiment 30, in which the temperature was high and specimens above medium size were used, there was better orientation with a greater intensity of avoiding reactions as is shown by the rating and per cent. of time spent in the halves of the pan. See Tables II. and III., and Chart II., graph 30. While in experiment 35 where the concentration was very high there was a falling off of the intensity of the avoiding reaction due to one specimen becoming more or less anæsthetized. See Table II. and Chart II., Expt. 35.

(*c*) *C. diogenes.*—In the acetic acid experiments as well as the carbon dioxide experiments the intensity of the avoiding reactions are rather low with the exception of experiment 31 which is rather high, the rating being 78. There was a stronger tendency to stop and then move forward and with longer periods of rest than was noted in the carbon dioxide experiments.

(*d*) *C. immunis.*—The avoiding reactions of *immunis* to acetic acid was definite in the time spent in the halves of the pan, by turnings and by turnings accompanied by backings. See Table II., Chart II., Expt. 37 and 34. The intensity of the reactions in the acetic acid as well as in the carbon dioxide was rather low as is shown by Tables I. and II.

3. *Hydrochloric Acid Gradient.*

After having completed the experiments with carbon dioxide and acetic acid it was thought advisable to test the crayfishes with some inorganic acid and thus determine the difference or similarity of the reactions of the crayfishes to the different acids and to better compare the reactions of the four species of crayfishes. Hydrochloric acid was selected for this purpose. Since the ion constant of hydrochloric acid in very weak concentrations is approximately one (Stiegletz, '11) very low, concentrations were used in all the experiments. By an inspection of the tables it is seen that the same intensity of avoiding reaction was obtained in the low concentrations of hydrochloric acid but of high ion concentration as was obtained by the higher concentrations of acetic acid and carbon dioxide of lower ion concentration. Not only was the intensity of the avoiding reactions high in proportion to the concentration, but the reaction of all four species was more definite as is indicated by the turnings from the ends and turnings accompanied by backings. See Table II. and Chart II. Expt. 47, Chart II. was extended over a period

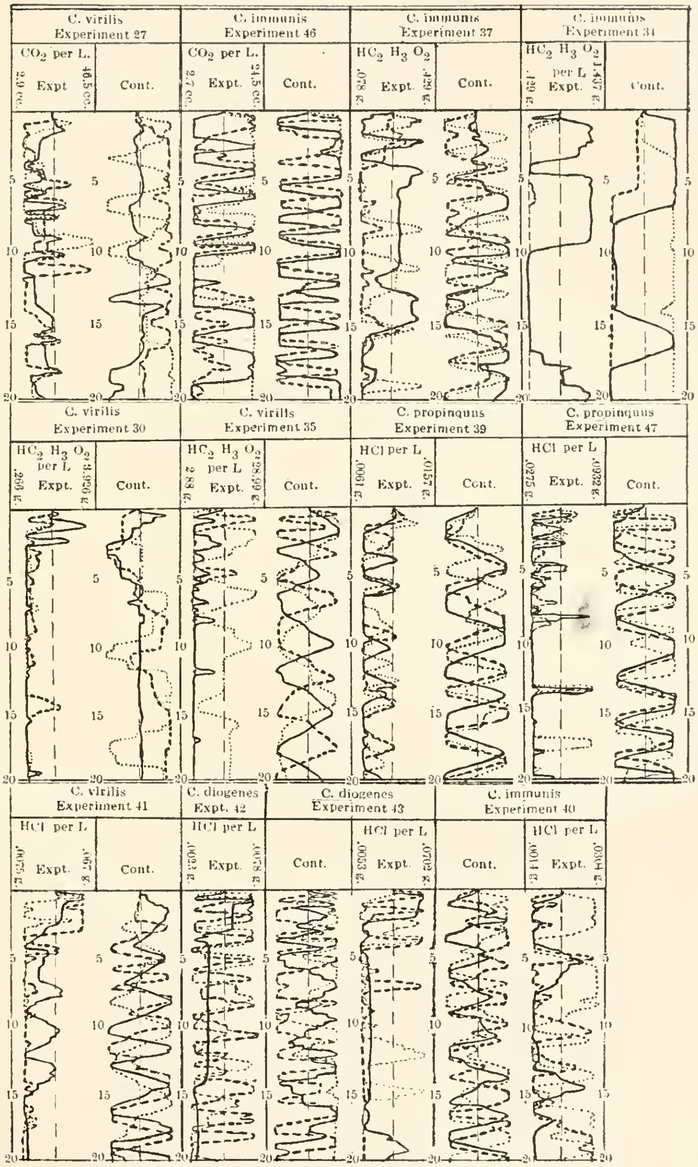

CHART II. Showing reaction of the crayfishes to gradients of carbon dioxide and acetic and hydrochloric acids. The horizontal distance represents the length of tank. The vertical distance represents time in minutes. Different tracings represent individual animals. In all experiments except experiments and controls 27 and 47 each animal was tested separately, 20 minutes each.

of forty minutes to determine the modification of behavior over a longer period of time. It was found that there was a similar rhythm of invasions of the high concentration end with a final coming to rest at the low acid concentration end.

4. *A Comparison of the Reactions of the Four Species of Crayfishes Tested.*

While there were noted specific differences in the reactions of the four species of crayfishes tested, all sensed the carbon dioxide and acetic and hydrochloric acids. Observations show that *propinquus* gives specific reactions and orients to a gradient of these substances, while *virilis* orients to a less degree; *immunis* to high concentrations, and *diogenes* to a still less degree to both high and low concentrations. Both *propinquus* and *virilis* which were tested at low temperatures were affected by carbon dioxide to the extent of intoxication, and *virilis* was affected more or less by the acetic acid. All species, so far as their avoiding reactions were noted, showed modifications of behavior and with *propinquus* there was a tendency to come to rest in the low end.

VI. General Discussion.

In reviewing the data it is seen that in each set of experiments all the types of behavior are increased in intensity with increase in the concentration of acid used, and the question as to the relation between the cause of the different types of behavior is suggested.

In the first place there must be a gradient before there can be orientation. This is in accord with Weber's law which states: "The increase of the stimulus necessary to produce an increase of the sensation bears a constant ratio to the total stimulus,"[1] *i. e.*, there must be a definite ratio between the increased intensity of the stimulus and the original stimulus before there can be a sensation of an increased stimulus. If the crayfishes were reacting in accordance with this law, the rating, which is the numerical expression of the degree of the reaction when both turnings and time preference are considered, should be in proportion to Weber's ratio, see Table III. By turning to the experiments with *propinquus* in carbon dioxide and comparing the ratios of the increased concentrations at the high acid concentration end with the concentration at the low end of the low temperature experiments, it will be seen that there is no definite relation between the two. This comparison may be objected to on the grounds that *propinquus* was intoxicated by the carbon dioxide, but by turning to the acetic acid experiment, where *propinquus* was not affected there is seen the same variation between the ratings and Weber's ratio. At the same time it will be seen that there is a more definite relation between the rating and the total concentration of the acetic acid. In general the lowest total concentrations of acids have the lowest ratings. Experiments 15 and 16 are exceptions but still the range is not wide as compared with the great variation of Weber's ratios.

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[1] James, The Principles of Psychology.

Now turning back to the carbon dioxide experiments (Table III., Expts. 2, 26 and 5) the intensities of the reactions are in reverse proportion to Weber's ratios, but are in direct proportion to the concentrations of the carbon dixoide solution used in the experiments. In the hydrochloric acid experiments (see Chart II. and Table III., Expt. 39 and 47) the intensity of the reactions conform with Weber's ratio, but at the same time it conforms with the concentrations of the hydrochloric acid used. Thus it is seen that the total concentration of the acid determines the intensity of the reaction. In other words the intensity of the reaction varies directly as the hydrogen ion concentration. This view is supported by comparing the ratings of the carbon dioxide and acetic and hydrochloric acid experiments. It is seen by comparing the carbon dioxide and acetic and hydrochloric acid experiments (see Table III.) that the rating on an average of the hydrochloric acid experiments are highest, acetic acid next and carbon dioxide lowest. This is due not to the higher molecular concentration of the hydrochloric acid over that of the acetic acid, since the molecular concentrations of the acetic acid were higher than that of the hydrochloric acid (Tables I. and II.), but to the higher ionization of hydrochloric acid over that of acetic acid and acetic acid over that of carbon dioxide in solution, thus giving higher ion concentrations.[1] These same points are suggested by the comparison of the ratings of each of the other three species in the hydrochloric acid, acetic acid and carbon dioxide experiments. It is interesting to note that when turnings only are considered that in all four species in the carbon dioxide, acetic acid and hydrochloric acid experiments that there is an increase of per cent. of turnings from the high acid concentration end over the low concentration acid end in the order named. Thus the hydrochloric acid again has the greatest intensity in avoidance of the high acid concentration end. These points are shown by the experiments with *propinquus* although the acetic acid experiments were of longer duration and were performed at a lower temperature than the hydrochloric acid experiments. The conclusion that intensity of negative reaction is directly proportional to the concentration of H ions can only be suggested, as sufficient data to warrant a definite conclusion are wanting.

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[1] Stieglitz's table of the ionization constants of acids, 1911.

The above suggestion might receive objection on the ground that there may be specific differences in the effect of the three acids used. Such an objection is supported by the fact that *propinquus*, in the low temperature experiment, is intoxicated by carbon dioxide and not by acetic acid. This apparent difference may, however, be explained on the ground that the carbon dioxide, since it diffuses more rapidly than the acetic acid, really produces a higher hydrogen ion concentration in the blood of the crayfishes than does the acetic acid, in spite of the fact that the latter acid is more highly ionized. The carbon dioxide would also tend to increase the carbon dioxide in the animal's blood by preventing the escape of the supply of this gas that is constantly being given off by the tissues of the animal. The acetic acid and hydrochloric acids would not offer any such hindrance to the diffusion of the internal gas into the water, and would not, therefore, be as detrimental as the carbon dioxide.

The periodicity and final cessation of invasion of the high concentration end of the experimental tank is a modification of behavior that may be brought about by increase in sensitiveness on the part of the crayfishes, or by a more rapid reaction to the same sensation. In the one case the cause is physiological, in the other the explanation must be psychological. If the modification is psychological then the animals must respond from associated memory. Shelford and Allee ('13, '14) have pointed out that it is hard to locate the things associated. Besides association formation is usually very slow for Yerkes ('08) states that 50 to 100 trials are necessary for the crayfish to form a perfect association in a simple labyrinth. The same slowness of modification would be expected of association due to a stimulus unless the sensitiveness of the animals was in some way progressively increased.

The modification is rapid, the number of invasions being sometimes but one before complete avoidance of the high end followed. This modification is probably due, as Shelford and Allee ('13) have pointed out, to increased sensitiveness on the part of the crayfishes and as they have further suggested, the greater sensitiveness may be the result of an increase in the hydrogen ion content of the blood of the animals.

In the cases where *propinquus* came to rest upon the screen in the low end and remained there for the rest of the experiment, the reaction may be considered the climax of the behavior modification, especially since the animals made this reaction more quickly in the presence of high total concentrations of acid than in the low. These points are shown by *propinquus*, Chart I., Expts. 5, 14. The explanation as to why the animals came to rest at all after being made more sensitive by the acid is not clear, but probably a combination of factors, one of which is thigmotaxis, were acting.

Shelford and Allee ('13) suggest carbon dioxide as a factor in determining the distribution of fishes and that the same may be true for crayfishes is suggested by the foregoing experiments. Crayfishes react to very weak concentrations of carbon dioxide and acetic acid and they were not overcome by the carbon dioxide except in concentrations higher than usually appear in natural waters. There seems also to be a correlation of the specific reactions of the two species with their respective habits. *Propinquus* is a pond form and its reactions were directive, while *virilis*, a rapid stream form, gave reactions which were much less directive. In natural waters carbon dioxide would be encountered in rather high concentrations by *propinquus* and that this species may react to these concentrations to its own advantage is indicated by the experiments heretofore described. *Virilis*, however, in its stream habitat would seldom encounter carbon dioxide concentrations of anything more than a very low degree and thus we find that this species does not react definitely to the gas. *Virilis*, then probably, while sensitive to carbon dioxide, orients itself in its environment by reactions to some other factor or factors. *Immunis* and *diogenes* are pond forms and are less sensitive to the acids but both avoid high concentrations. *Diogenes* although shown to be more sensitive than *immunis* orients less definitely as is shown by the per cent. of turnings from the high and low concentration acid ends. The specificities are again in coördination with the habitats of the species, *immunis* being a pond mud loving form, remaining in burrows only at times while *diogenes* remains almost wholly in burrows.

Summary.

1. Crayfishes sense the increase in carbon dioxide and acetic and hydrochloric acids in a gradient.

2. Both *propinquus* and *virilis* are intoxicated by carbon dioxide; *virilis* is also intoxicated by acetic acid but to a lesser degree.

3. The four species are susceptible to high concentrations of carbon dioxide and when subjected to high concentrations die in the following order, *virilis*, *propinquus*, *diogenes* and *immunis*.

4. *Propinquus* reacts negatively to the higher concentrations of carbon dioxide in a gradient, but when the total amount of acid present is large, the negative reaction may be interfered with by the direct detrimental effect of the acid.

5. *Virilis* reacts less definitely to the higher concentrations of carbon dioxide in a gradient than does *propinquus*. This is true whether the total concentration of the acid is large or small.

6. Both *diogenes* and *immunis* react more or less irregular to carbon dioxide due possibly to the lesser sensitiveness of these two species to this acid.

7. Both *propinquus* and *virilis* react negatively to the higher concentrations of acetic acid in gradients of this acid; *propinquus* reacts definitely in the presence of both high and low total concentrations; *virilis* reacts definitely to low total concentrations,

but not so definitely to high total concentrations; *diogenes* reacts irregular and less intense than the first two species while *immunis* reacts more definitely but with low intensity.

8. All four species react more strongly to hydrochloric acid than acetic acid and more strongly to acetic acid than carbon dioxide.

9. The intensity of avoiding reactions of all species to all acids tested as is shown by turnings only are in the following order; *propinquus*, *virilis*, *immunis* and *diogenes*.

10. The intensity of avoiding reactions of all four species varies directly as the total concentrations of the acids, and probably directly as the hydrogen ion concentration.

11. Rapid modification of behavior is shown by all four species. This modification may be due to the increased sensitiveness on the part of the animals, the increased sensitiveness being the result of higher ion concentration in the animal's blood.

12. The specific reactions of the crayfishes in gradients of carbon dioxide may be correlated with their habitats.

Acknowledgments.

I wish to express my appreciation and sincere thanks to Dr. V. E. Shelford, under whose direction this work was undertaken, for his constant help and many suggestions; also to Mr. M. M. Wells for assistance in determining the species of crayfishes used, and for suggestions during the performing of the experiments and writing of the manuscript.

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BIBLIOGRAPHY.

Abbott, C. C.
Notes on the Habits of Certain Crayfishes. Am. Nat., Vol. 7, pp. 80–84.

Bell, J. C.
'06 The Reactions of Crayfishes to Chemical Stimuli. Jour. Comp. Neur. and Psychol., Vol. 14, pp. 229–326.
'12 The Reactions of the Crayfishes. Harvard Psychological Studies, Vol. 2, pp. 615–644.

Chidester, F. E.
'12 The Biology of the Crayfishes. Am. Nat., Vol. 46, pp. 279–293.
'08 Notes on the Daily Life and Food of *Cambarus bartonius bartonii*. Am. Nat., Vol. 42, pp. 710–716.

Garman, Sam.

'89 Cave Animals from Southwestern Missouri. Bull. Mus. Comp. Zool., Vol. 17, pp. 225–240.

Harris, J. A.

'03 The Ecological Catalogue of the Crayfishes belonging to the Genus *Cambarus.* Kans. Univ. Sci. Bull., Vol. II., No. 3, pp. 51–184.

'01 Notes on the Habit of *Cambarus immunis.* Am. Nat., Vol. 35, No. 411, pp. 187–191.

Holmes, S. J., and Homuth, E. S.

'10 The Seat of Smell in Crayfishes. Biol. Bull., Woods Hole, Vol. 18, pp. 155–160.

James, William.

'93 The Principles of Psychology. Vol. I., p. 537.

Nagal, W. A.

'94 Geruchs- und Geschmackssinn. Bibliotheczoologica, Leuchart und Ghun., Vol. 18, pp. 1–207.

Pearse, A. S.

'10 The Crayfishes of Michigan. Mich. Geo. and Biol. Sur., Publication 1, Biological Series 1.

Putnam, F. W.

'75 Remarks on the Mammoth Cave and Some of its Animals. Proc. of the Soc. of Nat. Hist. Boston, Vol. 17, pp. 222–225.

Shelford, V. E., and Allee, W. C.

'13 The Reactions of Fishes to Gradients of Dissolved Atmospheric Gases. Jour. Expt. Zool., Vol. 14, No. 2, Feb. 1913.

'14 Rapid Modification of Behavior of Fishes by Contact with Modified Water. Jour. An. Behav. Vol. 4, No. 1, pp. 1–30.

Stieglitz, Julius.

'11 The Elements of Qualitative Chemical Analysis. Part I, p. 104.

Wells, M. M.

'13 The Resistance of Fishes to Different Concentrations and Combinations of Oxygen and Carbon dioxide. Biol. Bull. Vol. 25, No. 6, pp. 323–347.

Williamson, H. C.

'10 On the Larval Stages of Decapod Crustacea. 19th Report. Fish Board, Scotland, pp. 92–119.

Wright, R. R.

'84 Comparison of the Socalled Olfactory Organs of *Cambarus pellucidus* with those of *C. propinquus.* Am. Nat., Vol. 18, pp. 272–273.

Yerks, R. M., and Huggins, G. E.

'03 Habit Formation in the Crayfishes, *Cambarus affinis.* Harvard Psychological Studies, Vol. 1, pp. 565–577.

BREEDING HABITS OF THE HETERONEREIS FORM OF PLATYNEREIS MEGALOPS AT WOODS HOLE, MASS.

E. E. JUST.

Verrill ('73) first described *Platynereis megalops* figuring in his "Report" a male of the heteronereid phase. Later ('79) he figured the nereis-form and the female of the heteronereis-form changing the name he first gave, *Nectonereis megalops*, to *Nereis megalops*. Andrews, who ('91) had discovered the egg *Nereis limbata*, in a paper on the eyes of annelids speaks of the worm as *Nereis alacris*. I am indebted to Dr. J. Percy Moore who identified the animal as *Platynereis megalops*, Verrill. The belief seems to prevail that in the study of the cell lineage of *Nereis* ('92) Wilson indiscriminately used the males and females of *Nereis* and *Platynereis*. But this belief is by no means founded on any statement in Wilson's paper. Bonnevie ('08) has perhaps strengthened popular misconception through her descriptions of the "two varieties" of *Nereis limbata* at Woods Hole.

I. Swarming Habits.

The swarming of *Platynereis* is closely similar to that of *Nereis* (cf. Lillie and Just). There seems to be some variations as noted below. The behavior shows as that of many other forms a definite lunar periodicity: the sea-urchins (Tennent), the Japanese palolo (Izuka), the Pacific palolo (Woodworth and others), the Atlantic palolo (Mayer), *Amphitrite* (Scott), *Nereis dumerilii* (Hempelmann), etc.

Observations were made during the seasons of 1911, 1912 and 1913 at the Marine Biological Laboratory, Woods Hole, Mass. The swarming habits of *Platynereis* have not been worked out as fully as have been those of *Nereis limbata* (Lillie and Just, '13). In the first place during 1911 and 1912 attention was focused mainly on the swarming habits of *Nereis;* moreover, at all times the primary object in the collecting of *Platynereis* was for experimental study. Strict watch, however, was kept on these worms throughout the summers named. This is especially true for the summer of 1913; during June, July, and August I went out every night, giving attention wholly to *Platynereis* swarming.

The animals on swarming nights swim near the surface of the sea, the males invariably appearing first, the females later. The females rarely exceed fifteen, and indeed on some nights no females swim, while the number of males may be very large. Verrill ('73) says that the worms swim at noon. I have never noted this.[1] Hempelmann ('11) might lead one to think that *Nereis dumerilii* swarms early in the morning. I looked for this during August, 1913, but did not find *Platynereis* swarming before or at sunrise. The evening swarm may last two hours.

The small reddish males swim with great rapidity in an ever more narrowing circle within the patch of light thrown by the observer's lantern until the swarm is at its height. Here and there often at a greater depth than the males swims with slow and even laborious movements, the larger female, pale yellow in color with a thin dorsal line of green—the remnant of the empty gut. One cannot but suspect that the sex ratio in some way depends on the rate of movement: the females are easy prey for fish, the males must easily escape their enemies. The sex ratio of the captured animals must be also influenced by the fact that the females tend to keep further below the surface than the males. This is true of *Autolytus* to a marked degree as I have repeatedly observed. (So too, Andrews, '92, and Mensch.) Verrill, however, says of *Nereis limbata* that in their burrows "there are few males in proportion to the females"—as in the case of *Platynereis*, the reverse is true of these worms during swarming.

As the male comes in the vicinity of female he swims very rapidly in spirals tangential to the surface. They swim together and after copulation and egg-laying, the female slowly sinks from view.

The swarming occurs nightly throughout the months of July and August during the dark of the moon. From new moon to full moon, whether there be moonlight or not the animals do not swarm. Only mature animals swarm.

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[1] In July, 1914, I found spent males swimming during the day.

I have never taken this Heteronereid at Woods Hole earlier than June 29. In 1911 I remained at Woods Hole until September 18; I took no worms after August 24. For 1913, August 19 is the date of last capture.

The following tables selected from data of 1911, 1912 and 1913 give some idea of the lunar periodicity of the swarming:

TABLE I. 1911.

(Date of first capture, July 20.)

Moon Phase.	Date.	Number of Females.	Number of Males.
Full moon	August 8	0	0
	9	0	0
	10	0	0
	11	0	0
	12	0	0
	13	0	0
	14	0	0
	15	0	0
	16	1	6
Third Quarter	17	0	0
	18	0	0
	19	0	0
	20	0	0
	21	4	5
	22	6	6
New Moon	23	8	8
	24	10	10
	25	0	0
	26	0	0
	27	0	0
	28	0	0

Comparison with *Nereis* shows in the first place that the number of worms swarming is not so great. It was found, for instance, in collecting *Nereis* to be practically impossible to make an accurate estimate of the number of males; for that reason a record was kept of the females only. On two or three nights only did I find it impossible to estimate the number of *Platynereis* males swarming; on other evenings it was easily possible to count them. The swarm of males on the evening of August 11, 1912, was wonderful. For a few minutes the sea was alive with thousands of the rapidly swimming Heteronereids. In 1913 there was a similar swarm of females, but in no such numbers. As in the case of *Nereis* the collections were made in one place during the three years.

The season, moreover, appears to be shorter than that of

TABLE II. 1912.

Moon Phase.	Date.	Number of Females.	Number of Males.
	June 4 to July 2	None	None
	July 3	0	1
	4	0	0
	5	0	0
	6	0	0
Third Quarter	July 7	0	0
	8	0	0
	9	0	0
	10	1	3
	11	3	3
	12	3	3
	13	0	2
New Moon	14	2	2
	15	0	2
	16	0	1
	17	0	0
	18	0	0
	19	0	0
	20	0	0
First Quarter	21	0	0
	22	0	0
	23	0	0
	24	0	0
	25	2	1
	26	1	0
	27	0	1
Full moon	28	0	0
	29	0	0
	30	15	30
	31	2	30
	August 1	4	30
	2	3	30
	3	0	20
	4	0	0
Third Quarter	5	8	20
	6	14	20
	7	0	0
	8	12	10
	9	10	50
	10	12	100's
	11	15	1000's
New Moon	12	18	30
	13	0	0
	14	0	0
	15	3	6
	16	0	0
	17	0	0
	18	0	0
First Quarter	19	0	0
	20	0	0
	21	0	0
	22	0	0
	23	0	0
	24	0	0
	25	0	0
	26	0	0

TABLE II. 1912.—*Continued.*

Moon Phase.	Date.	Number of Females.	Number of Males.
Full moon	27	0	1
	28	0	1
	29	0	0
	30	0	0
	31	0	0

TABLE III. 1913.

Moon Phase.	Date.	Number of Females.	Number of Males.
	June 13–28	None	None
	29	0	1
	30	0	0
	July 2	0	1
		None	None
	17	0	1
Full moon	18	0	10
	19	0	30
	20	0	20
	21	1	25
	22	0	10
	23	0	8
	24	2	4
	25	50	30
Third Quarter	26	2	4
	27	5	3
	28	16	20
	29	5	8
	30	20	16
	31	21	8
	August 1	6	6
New Moon	2	1	20
	3	4	25
	4	0	16
	5	0	0
	6	0	1
	7	0	0
First Quarter	8	0	0
	9	0	0
	10	0	0
	11	0	0
	12	0	0
	13	0	0
	14	0	0
	15	0	0
Full Moon	16	0	2
	17	1	0
	18	0	1
	19	0	2
	20	0	0
	21	0	0

Nereis. In this my observations approximate those of Verrill. Also, the yearly swarming shows more variations than that of

Nereis. This is strikingly brought out by a study of the tables—especially when one recalls that I gave attention wholly to *Platynereis* for the year 1913. Curves of the runs of *Platynereis* would show that the heights tend to fall in with those of *Nereis*. The lunar periodicity is therefore more like that of *Nereis limbata* than that of *N. dumerilii* which in some respects *Platynereis* resembles.

II. Egg-Laying.

Males and females caught with a hand-net in the evening at the surface of the water and kept in separate dishes may be studied in the laboratory. If a male be transferred with a female to a dish of clean sea-water, the phenomena observed in the sea may be readily followed. The female packed eggs discernible through her pale thin body wall swims slowly in a straight line; or, with head bent at right angles to the body describes a circle of which the head is the center. The male swims in spirals tangential to the surface of the water. Soon his spirals are along the course of the female, her body finally becoming the long axis of his helical body. He entwines the female through this performance and straightens out, thus clutching her in the twist of his body. If this embrace be in the posterior region of the female's body, the male loosens slightly and pulls himself along the female's body. The task appears to be exacting. Often I have observed a rather small male that had worked himself forward after having grasped an unusually large female near the anal segment fall apparently too exhausted to complete the courtship. As the male slips along forward over the female, he lashes his tail back and forth. The female bends her head as if seeking the tail. If the female keep her body in a straight line, the male must move anteriorly until he entwines her body in the pharyngeal region. He now forms a coil around her head of which his tail is the apex. He thrusts his tail down into the coil of his own body and so into the waiting jaws of the female. The female is quiescent throughout. About six seconds after the female has received the anal segment of the male, the animals separate and eggs stream from the posterior segments of the female. The male may be held for a time by the female; if so he swims around, dragging her. I believe that the eggs escape, not through gonopores or the like, but through lesions of the body wall (cf. Scott.) Eggs escape from three or more posterior segments, occasionally from anterior segments. If escape by way of the posterior segments be experimentally inhibited, or if the female be slightly disturbed, the eggs seem to burst through the body wall at segments more anterior than otherwise. Females killed at the moment of oviposition show tears in the body wall.

After oviposition—and the whole process just described is in general the event of ten seconds—the female sinks to the bottom of the dish, a mere shred. In the laboratory placed in a little water it remains an irritable sticky mass for a time—incapable of exciting fresh males and finally dies, greatly shrivelled and blackened. Often, however, if flooded with fresh sea water it revives, expands to previous size, and swims around actively, almost perfectly transparent. I have kept these spent females alive for several hours. Since there are no sexual segments as in some annelids, but the whole body is little more than a locomotor ovary, it seems safe to assume that this egg-laying marks the end of the worm's existence.

Both animals must be in healthy condition for this behavior. Active males sometimes grasp females which because of rough handling in capturing are doubtless weak and fail to respond. The active males on the other hand are not very hardy: in the laboratory they rarely live twenty-four hours; one experiment made in 1913, failed to show any difference in the vitality of spent and unspent males. Normal females when placed in dishes with males fail to complete the courtship if the vitality of the male as by rough handling be impaired. Males and females may be kept in the same dish until death; if there be no courtship, there is no oviposition. Female *Platyneris* and male *Nereis* show no excitement when in the same dish, so male *Platynereis* and female *Nereis*. The male *Platynereis* ordinarily will embrace only an unspent female *Platynereis*. But on one occasion (July 23, 1913) all (8) males captured in turn and repeatedly embraced a *Nereis virens* eight inches long whose posterior segments had been lost. Once only I saw a male clutch a female which had extruded part of her eggs after a previous courtship.

The animals will go through this courtship when placed in a very dense suspension of India ink in sea-water; or total darkness. The reaction, therefore, cannot be due to sight. It is more likely due to some chemical emanation from the gravid female only since the spent female is not attractive to the male (Cf. F. R. Lillie on *Nereis*, '12, '13.)

A male *Platynereis* will embrace at least four females. On the evening of August 24, 1911, for instance, I put a male and a female in a dish. They swam around for a time, then the male wrapped himself about the female just back of the head, he let go, uncoiled himself, his tail remaining in the female's mouth. Immediately after release, he was placed with a second female; a minute later he induced oviposition. After intervals of five minutes he embraced a third and fourth female. In all cases the worms shed eggs. The male placed in fresh sea-water with an active female after an hour (11 P.M., about two hours in the laboratory after capture) failed to make a fifth clutch. Other males embraced two females. During 1912 and 1913 these observations were verified.

If after this egg laying behavior, both animals be removed from the dish or if the eggs be pipetted off as laid the eggs develop and normal swimming larvæ much like those of *Nereis limbata* result. If at the moment of her release by the male the female be put in a dish of clean fresh sea-water, eggs will stream out and subsequently develop.

In all these cases sperm are attached to the vitelline membrane within a hull of jelly which has been secreted through the breakdown of the cortical protoplasm of the egg. As in *Nereis* this jelly formation begins at the moment that the sperm touches the membrane. In *Platynereis* it is easily demonstrated that the inseminated eggs have this jelly when laid. Mechanical pressure either by the male, experimentally, or otherwise, as has been repeatedly demonstrated, will not induce oviposition. Mere clutching however recurrent—even by more than one male is not sufficient stimulus for oviposition. The head of the worm may be crushed—eggs will not escape; if she be cut in two, a few eggs escape. Only after thorough drying on filter paper or on sheer dry linen will the eggs burst through the body wall. If the female be finely minced in sea-water practically all the eggs may be procured. But eggs got in this way do not develop after insemination; they will not fertilize in sea-water. I have sections of uninseminated eggs killed after having remained upwards of two hours in sea-water; the cortical layer and the germinal vesicle are intact. (So *Nereis*.)

Eggs removed from worms after clutching only have the appearance of eggs from unembraced females—no sperm attached, subsequently no trace of development. Sperm are not found on the female's body (*e. g.*, hypodermic impregnation: cf. Whitman, Gardiner, etc.) or near the anus at the time of egg extrusion.

It appears, therefore, that mechanical stimulus is not sufficient to excite oviposition or sperm shedding. The eggs are not laid during or after the embrace nor are sperm shed unless the male's tail has been in the female's jaws. This, then, is a case of copulation followed by internal insemination. And indeed, the very elaborate and precise behavior indicates this. The sperm swallowed by the female inseminate the eggs in the body cavity, oviposition following immediately.

In 1911 gravid females before and after copulation were killed in Meves fluid but proved too refractory for cutting; in 1912, special precautions were taken. The following fixatives were used: Bouin, Gilson, 10 per cent. formalin, and Hennings mixture. With these mixtures the yolk and oil of the eggs are dissolved out, but the chitin of the jaws still makes the procuring of good sections difficult. In 1912 I thought that I had solved the difficulty when after experiments with various agents I procured with KCl, and KCN in sea-water eversion of the pharynx. But in 1913, these methods gave very indifferent results. Dissection of the jaws gave almost negative results. My best sections are those of July, 1912, killed in Gilson, Series A; those of August, 1912, killed in formalin, Series B, and those of 1913 kept in formalin for five months.

Sections of gravid females killed before courtship show no sperm in the body cavity. Sections of gravid females just after copulation show sperm among the antennæ, in the mouth, in the pharynx, and in the body cavity. The sperm may be traced, therefore, entering the mouth, passing down the pharynx whence they escape through lesions in the pharyngeal wall to the cœlom.

They may be found also attached to the vitelline membrane of the eggs. If one minces a male, one procures not only sperm but large numbers of corpuscles. Apparently, these are not injected into the female's body (cf. Scott on *Amphitrite*).

Since the mechanical pressure of the male, though often repeated, is not sufficient stimulus for egg-laying, it may be assumed that either the sperm or some secretion with, or of them stimulates in the female movements which bring about oviposition. In some cases males after having induced oviposition in two or three females cause egg-laying in a third or fourth as noted above. A slight amount of this substance, therefore if such there be in addition to the sperm themselves, is sufficient to initiate egg-laying. The injected substance, on the same ground could scarcely exert sufficient pressure to stimulate oviposition.

I had projected for 1913 various experiments to determine this point. The first experiment on the list, however, was clear enough to warrant abandoning the others. I put a female in a dish with no water. If a drop of sea-water be put on her head there is no response. Only complete drying causes breakdown of body wall. If instead of pure sea-water the minced female be added there is no response. But if a drop of minced male be added oviposition follows. This observation was made several times.

The following protocol from notes of the night of July 25, 1913, is typical:

Experiment.—Six males cut up in water adherent to their bodies (*i. e.*, not dried). Dried female put in this sperm suspension. No oviposition. A second female placed in the sperm suspension; and a third. No oviposition.

2. Three males cut up in three drops of sea-water. Two successive females used. No oviposition.

3. Six dried males cut up. Two dried females placed with heads in the sperm suspension. Eggs laid. Next day: trochophores.

4. Three males cut up in two drops of sea-water. Two dried females placed with heads in the sperm suspension. (Both females later copulated with males and laid eggs.) No eggs laid.

Oviposition, then, is clearly brought on through the ingesting of sperm with very little sea-water.

Nereis diversicolor O. F. Muller gives birth to living young. *Autolytus* (Agassiz) carries its larvæ in a brood pouch. In both of these forms there is probably internal insemination. Eisig has described copulation in an annelid, *Capitella*. *Platynereis* is of interest in that oviposition so quickly follows copulation.

III. The Nereis Form of Platynereis Megalops.

As in the case of *Nereis* an attempt has been made to rear the larvæ of *Platynereis*. Best results were obtained during 1913. A table was kept of the development of the young worms and their characteristics noted. They closely resemble the larvæ of *Nereis dumerilii* described by Hempelmann which he obtained from his cultures. Some of my worms aged six months measured four centimeters. It is hoped that a study of these forms will give a clue to the swarming habit.

LITERATURE REFERRED TO.

Agassiz, A.

'62 Alternate Generation in Annelids and the Embryology of the *Autolytus cornutus*. Boston Journal Nat. Hist., Vol. 7.

Andrews, A. E.

'91 Report on the Annelida polychæta of Beaufort, N. C. Proc. U. S. Nat. Mus., Vol. 14.

'92 Eyes of Polychaetous Annelida. Jour. Morph., Vol. 7.

Bonnevie, K.

'08 Chromosomenstudien. II. Heterotypische Mitose als Reifungscharakter. Arch. für Zellforsch., Bd. 2.

Eisig, H.

'87 Fauna und Flora des Golfes von Neapel. Monographie 16: Capitelliden.

Gardiner, Ed. G.

'98 The Growth of the Ovum, Formation of the Polar Bodies, and the Fertilization in *Polychoerus caudatus*. Jour. Morph., Vol. 15.

Hempelman, Fr.

'11 Zur Naturgeschichte von *Nereis dumerilii* Aud. et Edw. Zoologica, Bd. 25, Lief 1 (Heft 62).

Izuka, Akira.

'03 Observations on the Japanese Palola, *Ceratocephale osawai*, n. p. n. sp. Jour. Coll. Sci. Imp. U. of Tokyo, Vol. 17.

Lillie, F. R.

'12 The production of Sperm Iso-agglutinins by Ova. Science, N. S., Vol. 36, No. 929.

'12 Studies of Fertilization, V. The behavior of Spermatozoa of *Nereis* and Arbacia the special reference to egg-extractives. Jour. Ex. Zool., Vol. 14.

Lillie, F. R., and Just, E. E.

'13 Breeding Habits of the Heteronereis Form of *Nereis limbata* at Woods Hole, Mass. Biol. Bull., Vol. 24.

Mayer, A. G.

'08 The Annual swarming of the Atlantic Palolo. Publication 102, Carnegie Institution of Washington.

Mensch, P. Calvin.

'00 Stolonization in *Autolytus varians.* Jour. Morph., Vol. 16.

Scott, J. W.

'09 Some Egg-laying Habits of *Amphitrite ornata,* Verrill. Biol. Bull., Vol. 17.

Tennent, D. H.

'10 Variation in *Echinoid Plutei.* A study of variation under laboratory conditions. Jour. Ex. Zoöl., Vol. 9., No. 4.

Verrill, A. E.

'73 Report upon the Invertebrate Animals of Vineyard Sound and the Adjacent Waters, with an Account of the Physical Characters of the Region. U. S. Com. of Fish and Fisheries, Part I. Washington.

'79 New England Annelida, Part I. Historical Sketch with Annotated Lists of the Species hitherto recorded. Trans. Conn. Acad. Arts & Sci., Vol. 4.

Whitman, C. O.

'91 Spermatophores as a Means of Hypodermic Impregnation. Jour. Morph., Vol. 4.

Wilson, E. B.

'92 Cell Lineage of *Nereis.* Jour. Morph., Vol. 6.

Woodworth, W. Mc.

'07 The Palolo Worm, *Eunice viridis* (Gray). Bull. Mus. Comp. Zool., Vol. 51.

ON THE STRUCTURE OF THE INNER EAR IN TWO PRIMITIVE REPTILES.

E. C. CASE.

In 1885 Cope[1] described the structure of the brain and the inner ear of one of the cotylosaurian reptiles, *Diadectes sp.* In this paper he figured the structure of the canals and concluded as follows: "The result of this examination into the structure of the auditory organs in the Diadectidæ may be stated as follows: The semicircular canals have the structure common to all the Gnathostomatous Chordata. The internal wall of the vestibule remains unossified as in many of the fishes and a few batrachians. There is no rudiment of the cochlea, but the vestibule is produced outward and upward to the fenestra ovalis in a way unknown in any other family of the vertebrates."

Fig. 1 shows the arrangement of the semicircular canals as given by Cope.

Among the many specimens collected from the Brier Creek Bone-bed by the University of Michigan expedition to Archer County, Texas, in the summer of 1913 there are several complete and nearly complete basi-cranial regions of the Permian or Permo-Carboniferous reptiles, *Dimetrodon* and *Edaphosaurus*. The structure of the basi-cranial region in these forms has already been described by the author,[2] but the recovery of this new material makes it possible to describe the condition of the ear cavity.

The specimen of *Edaphosaurus*, No. 3446 University of Michigan, probably belongs to the species *cruciger* of Cope. The bones of the ear region are undistorted and the cavity shares in the perfection of preservation. This is shown by the complete correspondence of the two sides and by the similarity of the cavity to that in less perfectly preserved specimens. If the coossified elements which shelter the inner ear be held in the normal position, as shown in Fig. 2, it will be seen that there is the trace of a groove running apparently horizontally from without inward; at the inner upper corner of the cavity it joins the trace of a second groove running nearly vertically. These are the marks of the anterior and posterior semicircular canals and correspond to the horizontal antero-posterior and the vertical antero-posterior canals of Cope's figure. Where the two canals meet there is a projection upward and forward of the cavity which probably lodged a rudiment of the apex of the sinus utriculus superior (terminology of Retzius). At the outer ends of the canal there is evidence of slight enlargements which lodged the anterior and posterior ampullæ. Just below the point of union of the two canals there is an elevation of the cavity indicating that the sinus utriculus posterior was inclined inward and forward. The cavity for the utriculus is relatively large and higher than wide. On the outer side of the broken surface of the cavity, nearly opposite the middle point there is a slight excavation indicating the position of the external canal. At the lower end of the cavity there are two extensions, one running into the paroccipital (opisthotic) bone and parallel to its axis, this lodged the

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[1] Cope, *Proc. Am. Phil. Soc.*, p. 234, 1885.

[2] Case, Publication 55, Carnegie Institute of Washington, p. 98, 1907. Williston and Case, Publication 181, Carnegie Institution of Washington, p. 81, 1912.

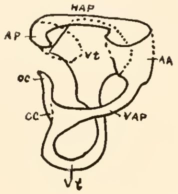

FIG. 1.

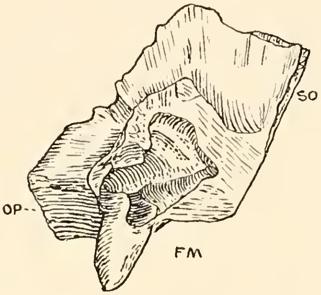

FIG. 2.

FIG. 1. Outline of the canals of the inner ear of *Diadectes* sp. From Cope. *hap*, horizontal antero-posterior canal; *aa*, anterior ampulla; *vap*, vertical antero-posterior canal; *vt*, vertical transverse canal; *ap*, posterior ampulla; *cc*, canalis communis of the vertical antero-posterior and the vertical transverse canals; *oc.os*, commune of the same.

FIG. 2. Inner view of the right otic region of *Edaphosaurus*, showing the cavity of the ear. *So*, supraoccipital; *op*, opisthotic; *fm*, foramen magnum. × 1.

lagena and shows no evidence of any curvature; the second extension is smaller and connected with the lagena cavity, it can only be for a considerable remnant of the sacculus.

The inner wall of the otic cavity was incomplete and the fenestra ovalis opened about opposite the upper end of the lagena.

The second specimen, No. 3447 University of Michigan, is that of one of the smaller but undeterminable species of *Dimetrodon*. It is as well preserved as that of the *Edaphosaurus*, both sides being present and identical in appearance and the details of the structure further verified by other specimens. The general arrangement of the canals and cavities is the same as in *Edaphosaurus* but it is smaller. The evidence for the presence of a apex of the sinus utriculus superior is less pronounced than in the first specimen but there can be no doubt of its presence. The two lower cavities, for the lagena and the sacculus, are narrower and in less open connection. The arrangement of the canals is shown in Fig. 4.

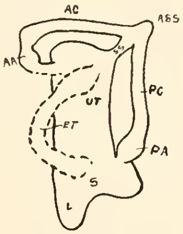

FIG. 3.

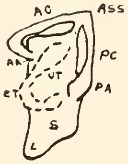

FIG. 4.

FIG. 3. Outline of the canals of the right side in *Edaphosaurus*. *ac*, anterior canal; *ass*, apex of the sinus urticule posterior; *pc*, posterior canal; *pa*, posterior ampulla; *s*, sacculus; *l*, lagena; *et*, external canal; *ut*, utriculus; *aa*, anterior ampulla. × 2.

FIG. 4. Outline of the canals of the right side in *Dimetrodon* sp. Lettering the same as in Fig. 3. × 2.

The author is unable to bring the structure as here made out into adjustment with the figures given by Cope for *Diadectes*. In comparing the structure of the inner ear of these primitive forms with that of modern reptiles certain archaic characters are recognized. The presence of an apex to the sinus utriculus superior is notably primitive, Retzius shows it as present in certain fishes but it is not noted in the amphibians and reptiles.

The straight lagena and the relatively large remnant of the sacculus is also primitive; a large sacculus occurs in the Amphibia Ecaudata and in some lizards, as *Lacerta viridis*. The canals are entirely within the otic cavity and do not penetrate any of the adjacent bones. The lagena of the crocodile is quite elongate and the soft structures are said to show something of a twisting, the lagena of these forms is relatively shorter and broader than that of the crocodile but, of course, nothing can be said of the soft parts. The cavity of the inner ear does not exactly reproduce the membranous ear as the soft parts are separated from the wall by the perilymph but the author believes that the figures given represent a very fair approximation to the true form and proportions of the membranous ear.

PRELIMINARY REPORT OF CROSSING TWO HEMIPTEROUS SPECIES, WITH REFERENCE TO THE INHERITANCE OF A SECOND EXCLUSIVELY MALE CHARACTER.

KATHARINE FOOT AND E. C. STROBELL.
Oxford.

Attention of cytologists has been centered during the past few years on the chromosome theory that claims to offer an explanation of sex-determination—a theory due to the discovery of certain morphological differences in the chromosomes of the males and females of many species. This discovery is responsible for the chromosome hypothesis of sex-determination—an hypothesis that awaits the test of experiment.

Recently (Foot and Strobell, '13) we published the results of some cross-breeding experiments undertaken with the aim of testing the above mentioned hypothesis that the factors determining sex are carried and distributed by definite chromosomes. The character selected to test this hypothesis was an exclusively male character in *Euschistus variolarius*—a distinct dark spot which is present on the genital segment of the male only, and we claimed that the method of the transmission of this spot should be an index of the method of transmission of the entire male genital segment. This exclusively male character—the genital spot—is a distinguishing feature of *Euschistus variolarius*, but is absent in *E. servus*. We therefore selected these two species as well adapted to test the function of the so-called sex-chromosomes in the transmission of this exclusively male character. Our claim that the method of transmission of the genital spot may be interpreted as an index of the transmission of the male reproductive organs themselves has not been accepted by two critics (Morgan and Doncaster) who have recently referred to our results. In reply to the criticism of these two advocates of the chromosome theory of sex-determination, we quote the following from a paper now in press (Foot and Strobell, '14*b*).

"After this paper was sent to press a notice of our results appeared in the following publications: 'Heredity and Sex,' Morgan ('13), and 'Chromosomes, Heredity and Sex,' Doncaster, *Q. J. M. Sci.*, Vol. LIX. ('14).

The latter disposes of our results in a footnote, as data irrelevant to a paper entitled "Chromosomes, Heredity and Sex—A Review of the Present State of the Evidence with Regard to the Material Basis of Heredity, Transmission and Sex-Determination."

From his report of the evidence he draws the following conclusion:

"The facts of sex-limited[1] transmission thus support the hypothesis that both ordinary Mendelian factors and the sex-determining factor or factors are borne by chromosomes," p. 511, and in the above-mentioned footnote he adds: "The recently published work of Foot and Strobell cannot be used as an argument against this proposition. They have shown (as was previously known in birds and moths) that a secondary sexual character in Hemiptera can be transmitted through the sex that does not show it; but the character was not sex-limited[1] in transmission; their results, therefore, have no bearing in the present discussion." As opposed to this decision we claim that the very fact that the genital spot is not linked with one of the so-called sex chromosomes is a point that calls for a satisfactory explanation by those who believe in sex-determining chromosomes, and our results cannot be cancelled by a dogmatic assertion that they have no bearing on the subject.

Morgan treats the facts with more consideration and attempts to give an explanation of them, though his explanation appears to us more as an attempt to excuse the facts than to explain them. Part of his explanation is merely a restatement of our conclusions, and the remainder is not in harmony with the facts.

We concluded that our results demonstrate that the spot can be transmitted without the X- or the Y-chromosome and Morgan accepts this as follows, "these results may be explained on the assumption *that the factors lie in other chromosomes than the sex-chromosomes*."[2]

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[1] Sex-limited is used by Doncaster in the sense that sex-linked is used by Morgan.

[2] The italics are ours.

We concluded that if one assumes (for the sake of the argument) that the spot factors are in a diploid pair of chromosomes, it becomes necessary to assume other factors outside the chromosomes. We called such hypothetical factors "inhibiting factors" and we said of them: "We are forced to admit that inhibiting factors—whatever they are—must be located outside the chromosomes, in the region of pure hypothesis."

Morgan appears to accept this, calling such hypothetical factors "*things in the cell*," and symbolizing them as A. B. C. He says "the result (or character) that a factor produces depends on its relation to *other things in the cell* (here A. B. C.)," and he adds, "We are dealing, then, not with the relation of X to S *alone*, but this relation in turn depends on the proportion of both X and S to A. B. C."

In the above-quoted paragraph he includes in his explanation the assumption of a relation between the spot factors and the X-chromosome, and this we believe is a part of his explanation which is not sustained by the facts. The spot can be transmitted directly from the male to his male offspring—and therefore this must be by the male-producing spermatozoön—(if there is such a thing) and the so-called male-producing spermatozoön has no X-chromosome. It is impossible to believe that in such cases the inheritance of the spot is dependent upon the relation of the spot factors of the sperm to the X-chromosome in the egg, especially if the cross is made with a pure *servus* egg. Morgan evidently thinks this is possible, however, for in his explanatory diagram he illustrates a cross between *E. servus* ♀ × *E. variolarius* ♂, a cross which we explained we were unable to attempt on account of scarcity of material.

His diagram, if assumed to be an explanation based on the facts of our experiments, is further in error in its illustration of the F_1 hybrids. In his simple Mendelian scheme all the F_1 hybrids are illustrated as typical heterozygotes and the fact is ignored that two out of the eleven of our F_1 hybrids are like *servus* in having no spot. If such a modification of the spot can be caused by "*other things in the cell*," it would seem that merely calling these "A. B. C." is no explanation of the results.

Morgan excuses his attempted explanation on the ground that we have failed to explain our results. We make no apology for this. We believe the duty of the scientist is to curb the natural temptation to force an explanation of individual results, for science to-day is overburdened by premature and undigested generalizations. We would aim rather to follow the example of those scientists who are willing patiently and conscientiously to collect data sustained by the hope that some day the facts may be of value.

Both Morgan and Doncaster class the genital spot of *variolarius* with the secondary sexual characters of authors and they therefore interpret our results as not having the bearing on the theories of sex-determination which we claim for them. Now our claim has been that the genital spot of *variolarius* is an integral part of the male genital segment—the structure of the female genital segment being such that the spot could not be present in this segment without changing the form of the segment itself—and we have claimed that therefore a study of the transmission of the genital spot should give a trustworthy indication of the method of transmission of the entire genital segment.

This claim that the method of transmission of the genital spot should be an index of the method of transmission of the genital organs of the male, has been completely justified by further work on these hybrids.

In the present paper we shall report the results from the study of the transmission of a second exclusively male character, and it seems to us that these results cannot be set aside as having no bearing on "Sex and Heredity," for this second exclusively male character is the male genital organ itself—the intromittent organ. The genetic results from our study of the genital spot of *variolarius* may be open to the criticism that as the spot is "not directly connected with the act of reproduction" it should be classed with the secondary sexual characters; but the intromittent organ is certainly free from such criticism and can be justly classed as a primary sexual character. In view of the fact that our results from the study of the transmission of the *variolarius* spot have been set aside on the ground that the spot is a secondary sexual character and therefore has no bearing on the problem of the determination of sex, it is necessary first to establish the claim that the intromittent organ can be classed with the primary and not the secondary sexual characters. This apparently ought not to be difficult, but a difficulty does arise owing to the fact that recent authors who have discussed secondary sexual characters have avoided defining them and have neglected to state wherein they are to be distinguished from the primary sexual characters.

According to Darwin '59 Hunter defines secondary sexual characters as follows:

"The term, secondary sexual characters, used by Hunter, applies to characters which are attached to one sex; but are not directly connected with the act of reproduction."

Darwin '86 adopts Hunter's classification of primary and secondary sexual characters; but shows that even such an apparently clear cut definition encounters difficulties. He says:[1]

"With animals which have their sexes separated, the males necessarily differ from the females in their organs of reproduction; *and these afford the primary sexual characters.* But the sexes often differ in what Hunter has called secondary sexual characters, which are *not directly connected with the act of reproduction;* for instance, in the male possessing certain organs of sense or locomotion, of which the female is quite destitute, or in having them more highly-developed, in order that he may readily find or reach her; or again, in the male having special organs of prehension so as to hold her securely. These latter organs of infinitely diversified kinds graduate into, and in some cases can hardly be distinguished from, those which are commonly ranked as primary, such as the complex appendages at the apex of the abdomen in male insects. Unless indeed we confine the term 'primary' to the reproductive glands, it is scarcely possible to decide, as far as the organs of prehension are concerned, which ought to be called primary and which secondary" (p. 253).

Morgan '13 also appears to accept Hunter's classification, for in his rather full list of secondary sexual characters he includes none that are "directly connected with the act of reproduction." He opens his discussion of secondary sexual characters as follows:

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[1] The italics are ours.

"The Secondary Sexual Characters."

"In the most highly evolved stages in the evolution of sex a new kind of character makes its appearance. This is the *secondary sexual character.* In most cases such characters are more elaborate in the male, but occasionally in the female. They are the most astonishing thing that nature has done: brilliant colors, plumes, combs, wattles, and spurs, scent glands (pleasant and unpleasant); red spots, yellow spots, green spots, topknots and tails, horns, lanterns for the dark, songs, howlings, dances and tourneys—a medley of odds and ends" (p. 26).

If we are to discard Hunter's classification, because it is found difficult to determine into which class some of the characters rightly belong, we should have to be dissatisfied with many classifications that are thoroughly well established.

If we limit the term "primary sexual characters" to the reproductive glands, it offers an escape from the difficulties in classifying the prehension organs, as Darwin has pointed out; but it would seem that greater difficulties are met by refusing to place the intromittent organ in the same group with the reproductive glands; and placing it in the group with characters so far removed from "direct connection with the act of reproduction" as, for example, Morgan's list of secondary sexual characters, "brilliant colors, plumes, combs, wattles, and spurs, scent glands (pleasant and unpleasant); red spots, yellow spots, green spots, topknots and tails, horns, lanterns for the dark, songs, howlings, dances and tourneys—a medley of odds and ends." The intromittent organ is not only "directly connected with the act of reproduction"; but it is as much a part of the sex of the individual as the reproductive glands themselves. Any one of the characters in Morgan's entire list of male secondary sexual characters could appear in the female without changing her sex; but the intromittent organ is as clearly indicative of the sex as are the reproductive glands themselves.

If a definite chromosome carries the factors for determining sex and it therefore carries the factors for the reproductive glands, it would seem logical to suppose that the chromosome carrying the factors necessary for the development of the male reproductive glands would also carry the factors necessary for the development of the intromittent organ which, when present, is functionally a necessary adjunct of the glands, and as indicative of the sex as the reproductive glands themselves. If we cannot accept the mode of transmission of the intromittent organ as an index of the mode of transmission of the reproductive glands, it would seem necessary to discard all structural features or other characters, which are distinctive of the gonads of a given species, such as their distinctions in size, form, color, etc., and assume that these characters, associated with the gland, have a different mode of transmission from the gland itself.

This would prevent any experimental test being applied to the chromosome theories of sex-determination and leave free scope for the wildest cytological speculations. If we should place the intromittent organ in the group of secondary sexual characters, because it has certain features in common with these characters we ought logically to place the reproductive glands themselves in the same group. For example, both these organs, in common with most of the secondary sexual characters, can be transmitted to the opposite sex—hermaphrodites appearing in forms that are normally sexually distinct. A case in point is Goodrichs's '12 interesting and important discovery of a male amphioxus in which 49 of the gonads were testes containing ripe spermatozoa and one was an ovary containing ripe ova. It may be urged that the intromittent organ is a secondary sexual character on the evidence that in the development of the embryo it appears much later than do the gonads—this indicating that the gonads are more fundamental and stable morphological entities. But there are facts opposed to this interpretation—Smith '10 found that when the spider crab is infected by the parasite sacculina, the testes can become so greatly metamorphosed that some of the cells may develop into ova and *the same testis* contain *both* ripe ova and spermatozoa.

It would seem that the division between primary and secondary sexual characters in common with almost all attempts at classification, has the objection that the line of demarcation is not, at all points, perfectly clear; but we believe, in spite of this, that we are justified in classing the intromittent organ as a primary sexual character and that the results from the study of the transmission of this organ may justly be claimed as an index of the method of transmission of the reproductive glands themselves.

Before giving these results we would express our great indebtedness to Professor Poulton and to Dr. Eltringham, of Oxford. We are indebted to Professor Poulton for his kind response to our wish to find an experienced entomologist in England who would be willing to study *E. variolarius* and *E. servus* with the aim of finding other characters than the genital spot that could be studied in the hybrids. He kindly suggested Dr. Eltringham, of Oxford, to whom we are indebted for the discovery that there is a marked specific difference in the intromittent organ of *E. variolarius* and *E. servus*. This discovery has made it possible for us to secure the results which are recorded in this paper.

RESULTS AND DISCUSSION.

The intromittent organs of *E. variolarius* and *E. servus* differ markedly in their length. We have dissected these organs from the genital segment of many of the parent species, and from all the hybrids, both of the F_1 and F_2 generations. These have been mounted and photographed at a magnification of 20 diameters, and all have been carefully measured at this magnification. The intromittent organ of *E. variolarius* varies in length between 85·5 and 106 mm., while that of *E. servus* varies between 146 and 182 mm. These measurements were made from 62 pure *variolarius* specimens, and from 62 pure *servus* specimens, the mean length of the intromittent organ of *variolarius* being 96·5 mm., and of *servus* 166·41 mm.[1]

Photos 1 to 4 show four typical intromittent organs of *E. variolarius*, these four varying in length between 94 mm. and 98·5 mm. Photos 5 to 8 show four typical intromittent organs from *E. servus*, these four varying in length between 158 mm. and 182 mm. Photos 9 to 12 show four typical intromittent organs of the F_1 generation derived from *E. variolarius* ♀ × *E. servus* ♂. These four vary in length from 122 to 132 mm. We have ten intromittent organs of this F_1 generation, nine of these being variable intermediates, and one like pure *variolarius*.

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[1] A discussion of the mean lengths of the hybrids and of the back-cross, and their bearing on the Mendelian type of inheritance, will be given in a later and more detailed report of these results.

Thus the type of intromittent organ characteristic of the species is transmitted through the female to her male offspring, and also directly by the male, and we may add that this is further proved by the back cross (F_1 ♀ × pure *variolarius* ♂). Thus the mode of transmission of this second exclusively male character is like that of the genital spot—*both* of these exclusively male characters being transmitted through the female as well as directly from the male—neither of these characters therefore being sex-linked.

Photos 13 to 20 show eight typical intromittent organs from the F_2 generation, these eight varying in length between 85·5 mm. and 140 mm. A few of these specimens (photos 13–15) show that the factors which determine the genital spot and those which determine the intromittent organ are not linked in inheritance (see below).

In our study of the transmission of the genital spot of *variolarius* (Foot and Strobell, '14*a*) we divided the hybrids into three groups—those having a genital spot like that of pure *variolarius*, those without a spot like *servus*, and those with a spot intermediate between these two extremes. In order to compare the results from the study of the two exclusively male characters—the genital spot and the intromittent organ—we have again grouped the hybrids into three classes, those having a length of intromittent organ like that of *variolarius*, those with a length of organ like that of *servus*, and those with a length intermediate between these two extremes.

By this grouping it is possible to compare the genital spot of each individual hybrid with the type of intromittent organ of the same hybrid, in order to determine whether these two exclusively male characters are linked in inheritance—to determine to what extent the two are associated in their transmission. Before discussing this point we shall summarize the points of agreement in the inheritance of the two characters.

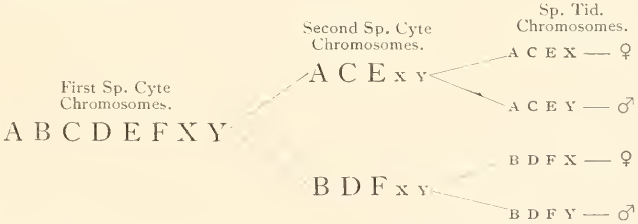
The intromittent organ—like the genital spot—is not sex-linked, this being shown by the facts that it is transmitted through the female, and also directly from the male to his male offspring. The intromittent organ—like the genital spot—is transmitted without the aid of either of the so-called "sex-chromosomes." It is transmitted without the aid of the Y-chromosome because it is inherited through the female, and it is transmitted without the aid of the X-chromosome because it is transmitted *directly* from the male to his male offspring. Like the genital spot, the intromittent organ fails to show dominance in the F_1 generation, and fails to show a simple Mendelian ratio in the F_2 generation; but the details demonstrating these facts must be reserved for our full report of this work, in which it will be possible to compare the inheritance of these two exclusively male characters in every individual of the F_1 and F_2 generations.

As in the case of the genital spot, we are forced to conclude that if the factors determining the inheritance of the intromittent organ are carried by definite chromosomes, they must be in *at least* a pair of diploid chromosomes, and as in the case of the genital spot, we are further forced to conclude that there are factors in the cell, outside the chromosomes, which determine just how many of the factors determining the character of intromittent organ shall find expression in the first and second generations of hybrids. The facts show, as in the case of the genital spot, that this cannot be accomplished by the assumed mechanism of division of the chromosomes, but is dependent upon hypothetical factors outside the chromosomes, and thus the distribution of unit factors through the mechanism of chromosome division seems to be an unnecessary assumption. If the factors essential to produce these two exclusively male characters are confined to one chromosome they can be in the Y-chromosome *alone*, for according to the mechanism of the two maturation divisions this is the *only* chromosome that can be in *all* the so-called male-producing spermatozoa. The facts, however, demonstrate that not only the genital spot, but the intromittent organ, can be transmitted without the aid of the Y-chromosome. If, as the facts demand, the factors for these two exclusively male characters cannot be in less than a pair of chromosomes, there seems no adequate reason for confining them to a single pair, or even to the chromosomes at all, for if factors outside the chromosomes and outside the mechanism of the division of the chromosomes, are responsible for the exact expression or total suppression of these characters, this deprives the chromosomes of a most important function which has been attributed to them, based on the mechanism of their division.

The results from the back cross demonstrate that the type of intromittent organ distinctive of the species can (like the presence or absence of the genital spot) be transmitted by *both* the so-called male-producing and female-producing spermatozoa. The back cross demonstrates not only that the male can directly transmit the intromittent organ distinctive of *variolarius;* but that the type of intromittent organ distinctive of *servus* is transmitted by the F_1 female, and therefore was transmitted by the female-producing spermatozoön of the first cross. If such a primary sexual character of the male can be carried by the female-producing spermatozoön, it is only logical to believe that female primary sexual characters also can be transmitted by both types of spermatozoa (male-producing as well as female-producing)—for it is difficult to believe that male and female primary sexual characters differ fundamentally in their method of transmission. The facts appear to deprive the male-producing spermatozoön of its distinctive function, and challenge the logic of endowing slight morphological differences in structures of the cell with causal attributes of fundamental importance.

Linkage in Inheritance.—If factors which stand for a given character are carried by a definite chromosome or pair of chromosomes, and the inheritance of the character is due to a special distribution of the factors at mitosis, it would seem logical to expect that the factors of two characters showing a very special mode of distribution (*i. e.*, exclusively male characters) would be contained in the same chromosome, and that this would be indicated by their being linked in the hybrids. We would expect the absence or presence of the genital spot, distinctive of one species, to be associated in inheritance with the type of intromittent organ characteristic of the same species. Even if the extent to which a character appears is dependent upon hypothetical factors outside the chromosomes, we would expect these hypothetical factors to act equally on two characters which are so closely associated as to be contained in the same chromosome. We should expect the two characters never to be so entirely dissociated that we find, in the same individual, the absence of spot characteristic of one species, associated with the type of intromittent organ distinctive of the other species. Instances of such complete dissociation do however occur and are shown, for example, in photos 13 and 14, which have the length of intromittent organ characteristic of *E. variolarius* (85·5 mm. and 99 mm.) while the specimens from which these organs were dissected have the *E. servus* absence of spot. Photo 15 has a length of intromittent organ almost equal to *E. servus* (140 mm.), while the specimen from which this was dissected has the genital spot distinctive of *E. variolarius*. There are, however, instances of association in the inheritance of the two characters, the intromittent organ and genital spot typical of one of the species occurring in the same F_2 individual; but exact classification of the full results shows that the two characters are transmitted quite independently of each other. The intermediates, having a large range of variation, make it possible for many of them to appear to show the two characters in the association that would be in harmony with the chromosome hypothesis, but an exact comparison shows that two plus and two minus intermediates are quite as frequently associated as are a plus and a minus intermediate. If we find such independence in the transmission of the two characters, there seems no logical reason for assuming that their factors are carried by the same chromosome. If their frequent independence in transmission forces us to locate them in at least two of the seven chromosomes contributed by each parent, there seems no adequate reason for confining them to the chromosomes at all, especially as their final mode of expression is not dependent upon the distribution at mitosis of unit factors carried by the chromosomes, but upon hypothetical factors outside the chromosomes.

As the advocates of the sex-determination theory may dismiss these results—as in the case of the genital spot—on the ground that the intromittent organ is not sex-linked, and is merely a secondary sexual character, it may be profitable to attempt to follow theoretically the transmission of what must be admitted are *primary sexual* organs, (the ovaries and the testes), while assuming that they are "sex-linked." This has its difficulties in the case of the testes, for the factors determining the sex-linked characters of authors are assumed to be in the chromosome which is homozygous in one sex and heterozygous in the other sex. This would place sex-linked factors for the testes in the X-chromosome of these insects; and locating these factors in this chromosome would involve their being in the female-producing spermatozoa only, and this type of spermatozoa therefore would carry the determining factors for *both* the ovaries and the testes, while the male-producing spermatozoa would carry neither the one nor the other. We shall try to show by an analysis of the chromosomes that it is as impossible to associate the testes with the sex-chromosomes as we have shown by experiment is true for the two other exclusively male characters—the genital spot and the intromittent organ. In order to simplify the discussion, we reproduce the following diagram used in an earlier paper to show the method of division of the 14 somatic chromosomes which are distinctive of both *E. variolarius* and *E. servus*. In the diagram we have used the method of designating univalents by the letters of the alphabet, bivalents being represented by AB, CD, EF.



Scheme of the two maturation divisions of *Euschistus variolarius* and *Euschistus servus* based on the assumption that the first maturation division separates autosomes of maternal and paternal origin and the second division halves them. The *XY*-chromosomes on the contrary being halved in the first division and separated in the second division. The relative positions of the autosomes may be changed unless definite chromosomes are always destined to the same pole, but reversing their position in this regard does not alter the end result—that the only chromosome common to both so-called male-producing spermatids is the *Y*-chromosome.[1]

The above diagram demonstrates the two types of spermatozoa, those having the X-chromosome—so-called female-producing; and those having the Y-chromosome—so-called male-producing. If we accept Morgan's conclusion that factors determining all sex-linked characters are located in the X-chromosomes and we assume that the primary sexual characters are sex-linked, this involves placing the factors determining both the ovaries and the testes in the X-chromosomes and, as stated above, this would effectually deprive the so-called male-producing spermatozoa of an essential male-producing function. This is so evidently out of harmony with the chromosome sex-determination theory, that it needs no further comment.

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[1] Only six of the twelve autosomes are designated.

If we attempt to place the factors determining the testes in the other sex-chromosome (the Y-chromosome) we meet difficulties that are equally obvious, for there are many forms that have no Y-chromosome at all. If we could ignore this important fact, we would have, in these insects, quite a diagrammatic demonstration of the chromosome sex-determination theory, for the Y-chromosome is the *only* chromosome that is in *all* the male-producing spermatozoa, just as the X-chromosome is the *only* chromosome that is in *all* the female-producing spermatozoa. Each is the *only* chromosome which is distinctive of the type of spermatozoa which it identifies. But the fact cannot be ignored that the Y-chromosome, so conspicuous in these insects, is absent in most forms, and we must therefore dismiss the possibility that the factors determining the testes of these insects are carried by this chromosome. The association therefore between the testes and the sex-chromosomes can be no closer than we have shown by experiment to be the case between the sex chromosomes and the other two exclusively male characters—the genital spot and the intromittent organ.

Realizing that the Y-chromosome cannot logically function as the carrier of the factors determining a male, Morgan '11 suggested that "the factors for producing the male must be in some other chromosome" (than the Y- or the X-chromosomes). We would consider this suggestion in relation to the factors determining the testes of these insects, ignoring for the present the fact that in his diagram illustrating this suggestion, Morgan does not place these factors in *one* chromosome but in a pair of chromosomes. If we attempt to place the factors for the testes in one of the autosomes, we meet difficulties that are quite as obvious as the difficulties in attempting to place the factors in the sex-chromosomes.

The above diagram (text Fig. 1) shows that the spermatozoa can be classed not only into two types (the so-called male-producing and female-producing), but each of these groups can again be separated into two types, in relation to their autosome content —one type containing the autosomes A, C, E, and the other type the autosomes B, D, F. As we are discussing the transmission of the testes, the factors for which are presumably carried by the male-producing spermatozoa, we shall consider the two types of these spermatozoa only—those with A, C, E, Y, and those with B, D, F, Y.

Our problem, as stated above, is to determine whether it is possible to place the factors which determine the testes of these insects in one of the autosomes.

A glance at text Fig. 1 shows that each of the autosomes is in *only half* the male-producing spermatozoa, and is also in half the female-producing spermatozoa. If, for example, we assume that autosome A carries the factors for determining the testes, and the X-chromosome carries the factors for determining the ovaries, we shall have *all* the female-producing spermatozoa carrying the factors for determining the ovaries, and in addition to this, half of these spermatozoa will carry the factors for determining the testes, as half of them have the A autosome. The male-producing spermatozoa, on the contrary, will not only carry none of the factors for determining the ovaries, but only half of them can carry the factors for determining the testes, as only half of them have the A autosome.

These conclusions, forced by an analysis of the chromosomes, are by no means in harmony with the demands of the chromosome sex-determination theory—thus it is quite as impossible to confine the factors for the testes to a single autosome, as we have shown is the case with the other two exculsively male characters —the genital spot and the intromittent organ. We might avoid this difficulty by assuming that the maturation divisions of one pair of the autosomes is like that of the XY-chromosomes, and that the factors for the testes are carried by one member of this pair. This involves, however, the further assumption that this autosome must follow the lead of the Y-chromosome or it might arrive in the female-producing spermatozoön. Unless we are willing to make some such unwarranted assumptions, it does not seem possible to make the association between the testes and the sex-chromosomes any closer than we have shown by experiment is the case between these chromosomes and the genital spot and intromittent organ. Those who would place these factors in the chromosomes must concede that they must be in *at least* a pair of autosomes; there seems indeed no reason for assuming a different mode of transmission for the testes than for the other exclusively male characters—the genital spot and the intromittent organ. We feel we are therefore justified in our claim that the mode of transmission of the genital spot and intromittent organ is an index of the mode of transmission of the reproductive glands themselves, and that our cross-breeding experiments offer direct evidence against the chromosome theory of sex-determination.

If we reconsider Morgan's suggestion that "the factors for producing a male must be located in some other chromosome" than the X-chromosome, and we interpret "some other chromosome" as a pair of autosomes (as Morgan does in his formula) this would locate the factors for the testes in this pair of autosomes and be quite in harmony with our conclusions, that factors for exclusively male organs, if carried by chromosomes, cannot be in less than a pair of chromosomes—and it supports our claim that the method of transmission of the genital spot and intromittent organ is an index of the method of transmission of the testes.

Morgan's formula

Gametes of female—X M—X M
Gametes of male—X M—M.

gives the female zygotes (X M + X M) just as many factors for "producing a male" (M M) as it gives for producing a female (X X) but he does not tell us what determines that the X gametes shall predominate.

According to Morgan's formula the term "female-producing spermatozoön" (X M) would appear to be a misnomer, for it carries "the factors for producing a male" as well as the factors for producing a female. The male-producing spermatozoön, on the contrary, carries the factors for producing a male only (M). The female-producing spermatozoön therefore can transmit exclusively male characters, as we have shown is the case, but the male-producing spermatozoa cannot transmit exclusively female characters. That exclusively female characters and exclusively male characters should have such a different mode of transmission does not appear to us to be a logical conclusion, but it is a question that it is possible to put to the test of experiment.

The point of view of the investigator as to the chromosome theory of sex-determination seems to be entirely dependent upon the extent of his belief in the individuality of the chromosomes. In these insects, for example, the so-called male-producing spermatozoa have the Y-chromosome and not the X-chromosome, and those who believe in such a degree of individuality of the chromosomes as is demanded by the chromosome hypothesis of sex-determination, must hold that a so-called male-producing spermatozoön *must* develop into a pronucleus with a Y-chromosome and never an X-chromosome. They must hold that the Y- and X-chromosomes are as individual as the king and queen of chess for example.

On the other hand, the cytologist who believes that the chromosomes, like other organs in the cell, are the expression rather than the cause of cell activities, can also believe that there are forces outside the chromosomes that determine whether an egg shall develop into a male or female and can further believe that these forces, acting on the developing pronucleus can cause its chromatin content to develop into the chromosome configuration which is demanded by the sex. As it is impossible to follow the metamorphosis of a spermatozoön into a pronucleus, the cytological proof can probably never be achieved; but there is definite evidence that cells which normally produce certain organs, can be forced by experimental manipulation to produce other organs which have quite different functions, and such a change of function must create a corresponding change of structure, not only in the visible final result, but in the initial changes of the cell itself. Thus we believe that the structure of the cell, or any part of the cell is not the determining factor, but is merely an expression of other forces.

BIBLIOGRAPHY.

Darwin, Charles.

'59 The Origin of Species.

'86 The Descent of Man.

Doncaster, L.

'14 Heredity and Sex—A Review of the Present State of the Evidence with Regard to the Material Basis of Heredity, Transmission and Sex-Determination. Q. J. M. Sci., Vol. LIV.

Foot, K., and E. C. Strobell.

'13 Preliminary Note on the Results of crossing two Hemipterous Species with Reference to the Inheritance of an Exclusively Male Character and its Bearing on Modern Chromosome Theories. Biol. Bull., Vol. XXIV., No. 3.

'14a Results of crossing *Euschistus variolarius* and *Euschistus servus* with Reference to the Inheritance of an Exclusively Male Character. Proc. Linn. Soc. London, Vol. XXXII.

'14b The Chromosomes of *Euschistus variolarius*, *Euschistus servus* and the Hybrids of the F_1 and F_2 Generations. Arch. für Zellf., Bd. 12.

Goodrich, Edwin S.

'12 A Case of Hermaphroditism in Amphioxus. Anat. Anz., Bd. 42.

Smith, Geoffrey.

'10 Studies in the Experimental Analysis of Sex. Q. J. M. Sci., Vol. 55, Pt. 2.

Morgan, Thomas Hunt.

'13 Heredity and Sex.

'11 An Attempt to Analyze the Constitution of the Chromosomes on the Basis of Sex-limited Inheritance in Drosophila. Jour. Exp. Zoöl., Vol. XI., No. 4.

Description of Plate I.

The length of the intromittent organs was measured by a small pair of architect's dividers fitted with No. 9 needle points, and the dividers were frequently tested by measuring a 100 mm. line. Each intromittent organ was photographed at a magnification of 20 diameters, and the measuring was simplified by numbering each division of 20 mm. by a pencil mark. The measurements were taken from the distal end of the intromittent organ at the point where the coil enters the gland, the coil being easily dissected off at this point (photos 3, 4, etc.).

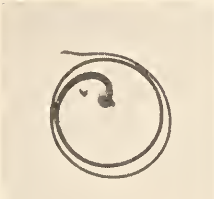
In photos 1, 2, 7, 9, a small part of the gland itself is retained. When part of the intromittent organ that is within the gland is preserved, the point from which the measurement was taken is easily determined, for the part within the gland is transparent and tapers at this point to a much smaller canal, *e. g.*, photos 15 and 19.

Photos 1 to 4. Typical specimens of the intromittent organ of *Euschistus variolarius*. Length of the organ of photo 1, 98·5 mm. Photo 2, length 97·5 mm. Photo 3, length 95 mm. Photo 4, length 94 mm.

Photos 5 to 8. Typical specimens of the intromittent organ of *E. servus*. Length of the organ of photo 5, 182 mm. Photo 6, length 158 mm. Photo 7, length 170 mm. Photo 8, length 162 mm.

Photos 9 to 12. The intromittent organs from four of the F_1 hybrids. Length of the organ of photo 9, 124 mm. Photo 10, length 132 mm. Photo 11, length 122 mm. Photo 12, length 126 mm.

Photos 13 to 20. The intromittent organs from eight of the F_2 generation. Length of the organ of photo 13, 85·5 mm. Photo 14, length 97 mm. Photo 15, length 140 mm. Photo 16, length 128 mm. Photo 17, length 147 mm. Photo 18, length 100 mm. Photo 19, length 124 mm. Photo 20, length 124 mm.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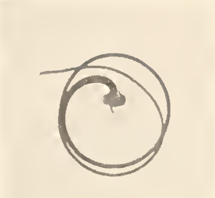
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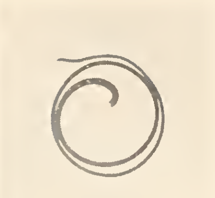
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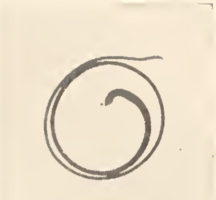
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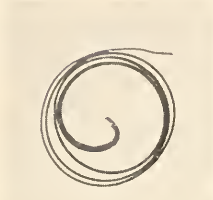
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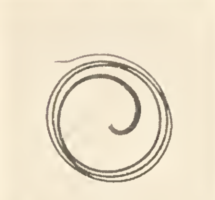
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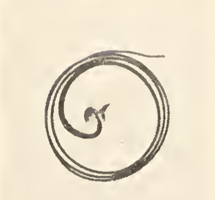
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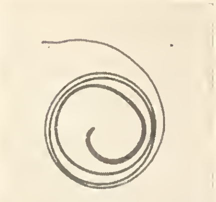
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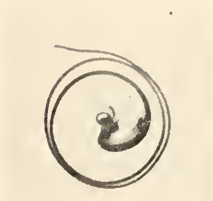
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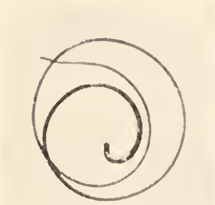
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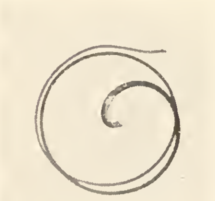
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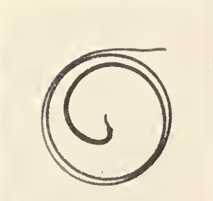
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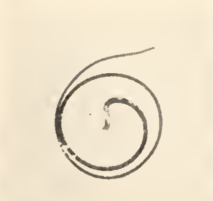
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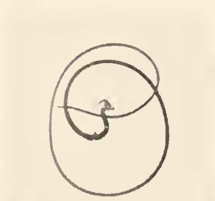
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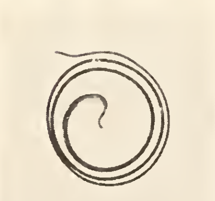
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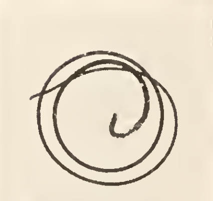
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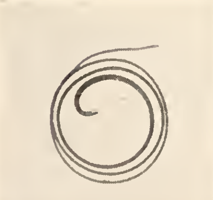
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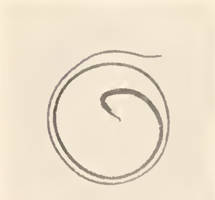
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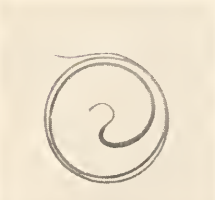
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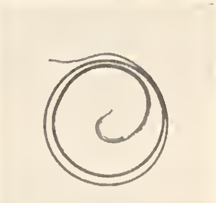
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BIOLOGICAL BULLETIN

IS THE FERTILIZATION MEMBRANE OF ARBACIA EGGS A PRECIPITATION MEMBRANE?

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In 1912 McClendon ('12) suggested that the fertilization membrane of sea urchin eggs is a precipitation membrane formed when two colloids of opposite electrical charge meet, namely the negative egg jelly (mucous, zona pellucida) and a positive substance secreted by the egg. Elder ('13) has advanced a similar view and recently McClendon ('14) has restated his former opinion. Of course the test of the theory is perfectly simple. An unfertilized egg from which the jelly has been removed should form no membrane when fertilized, and both McClendon and Elder state that this is the case.

I have utterly failed to confirm these statements and find that whether an egg forms a normal membrane or not is absolutely independent of the jelly which surrounds it. My method of determining this is as follows:—Eggs from one female are divided into two parts and the jelly removed from the eggs of one part by shaking two or three times in a test-tube and washing with sea water. Eggs of both lots are placed together upon a slide and india ink suspension, sperm, and, after mixing, a cover glass is added. It is perfectly easy to see which eggs are surrounded by jelly and which are not. All of them, whether with or without jelly, form fertilization membranes which in the two-cell stage surround the whole egg and are quite distinct from the hyaline plasma layer which is close to each blastomere. An egg without jelly touching the jelly of another egg should form a membrane, according to McClendon's idea, only on the side toward the jelly. Yet such a condition is never observed, but instead a membrane forms about the whole egg. Thus eggs without jelly will form fertilization membranes.

If *Arbacia* eggs are allowed to stand for about 52 hours at a temperature of 22° C. or for 3 to 4 days at a temperature of 12° C. no membranes form despite the fact that they may still be surrounded by a copious amount of jelly. This observation so far as I am aware was first made by Loeb ('03) and confirmed by myself ('10) and F. R. Lillie ('14). In the two-cell stage the blastomeres are widely separated because not surrounded by a fertilization membrane although the hyaline plasma layer is clearly visible.[1] Thus even an egg surrounded by jelly may fail to form a fertilization membrane.

I at first thought that McClendon's observations were due to the fact that he took so long a time in removing the jelly, (agitation and washing for "not more than 24 hrs.") that the mere age of the eggs would account for their inability to form membranes. I find, however, that the eggs *with jelly* must stand for over 52 hrs. at 22° C. before they are unable to form a fertilization membrane.

This fact suggests that the membrane-forming substance (membranogen) which passes out of the egg to form the membrane gradually diffuses away or is used up when the egg is allowed to stand. Since the membranogen is probably a protein we should expect it to diffuse away from the eggs without jelly much more readily than from those eggs with jelly. It is well known that colloids do not readily diffuse through each other. Such is actually the case and in this point lies, I believe, the explanation of McClendon's results. If we take the eggs from one female, remove the jelly from one lot by shaking, but allow it to remain on the other lot, both lots will form perfectly normal membranes if fertilized immediately. If both lots are allowed to stand for 24 hrs. and are then fertilized the eggs which have stood without jelly form no membranes while those with jelly form membranes only slightly thinner than normal. Membrane formation is not, then, a question of presence or absence of jelly but is dependent on the time the eggs have stood. We may imagine this to be due to the loss of some membrane-forming material from the eggs which much more readily takes place when the mechanical hindrance of the jelly is removed.[1]

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[1] The hyaline plasma layer appears much thicker than in freshly fertilized eggs and in my previous paper ('10) I described this as a special type of membrane. However there is nothing present at all comparable to the normal fertilization membrane and without quibbling over minute distinctions we may safely class these eggs as "without membranes." McClendon ('14) misquotes me in stating that I believe the fertilization membranes to be present on unfertilized eggs and to be simply lifted off after fertilization. I have held exactly the opposite view (Harvey, '10).

These experiments point against the view that the fertilization membrane is a precipitation membrane.

BIBLIOGRAPHY.

Elder, —.
'13 Arch. f. Entwick., 35, 195.
Harvey, E. N.
'10 J. Exp. Zool., 8, 365.
Kite, G. L.
'12 Science, n. s., 36, 562.
Lillie, F. R.
'14 J. Exp. Zool., 16, 550.
Loeb, J.
'03 Pflüger's Arch., 365, 59.
McClendon, J. F.
'12 Science, n.s., 33, 387.
'14 Zeit. f. physiol. chem. biol., 1, 163.

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[1] According to Kite ('12) membrane formation is merely the swelling of a fine invisible (unless stained) vitelline membrane together with a change of its optical properties. If that is true the inability of eggs which have stood for some time without jelly to form fertilization membranes would seem to be due to a loss, through solution, of the vitelline membrane.

SO-CALLED PARTHENOGENESIS IN THE WHITE MOUSE.

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In atretic follicles of ovaries in a number of different mammals the oöcytes go through a process which somewhat resembles maturation. Various stages of mitosis are seen and frequently a first polar body is present. In some cases the oöcyte is found divided up in a number of small parts some of which contain nuclei. This process has been described as a beginning of parthenogenetic cleavage and also as degenerative fragmentation. Bonnet ('00) gives a review of the work done up to that time and after considering all the evidence decides that the mitotic figures seen in such egg-cells are not those of parthenogenetic cleavage but are rather those of more or less abnormal maturation stages. Newman ('13) reviews briefly the work done since Bonnet's paper and presents the results of his studies on the armadillo in support of the view that "a limited amount of parthenogenetic cleavage occurs but that development proceeds no farther than two or three cell divisions." Van der Stricht ('01), who worked on the bat claims that in that form is found a beginning of true parthenogenesis. Rubaschkin ('07), who studied the guinea-pig, and Athias ('09), for the dormouse, state that the phenomena are to be interpreted as degenerative fragmentation which at the most merely resembles parthenogenesis.

In material which I have been preparing for a study of oögenesis in the white mouse I found that in the ovaries of young mice approaching sexual maturity there is a very extensive degeneration of follicles, most marked between the ages of twenty-five and forty days. As the work on this problem has all apparently been done on the ovaries of fully or young adult mammals, it was thought worth while to use the material in these immature ovaries[1] for a study of this so-called parthenogenesis.

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[1] The material used for this work consists of ovaries of white mice varying in age from twenty to ninety days. These ovaries were fixed in Carnoy's fixer

In the white mouse, it is certain that the changes taking place in the oöcytes are in some way correlated with the atresia of the follicles, for in follicles which have not begun to degenerate the egg-cells are normal in appearance and the nuclei are normal resting nuclei. But in follicles overtaken by atresia the cytoplasm of the oöcytes is found to stain more deeply with acid stains, fat granules are found in large numbers, and the nuclei are in various stages of mitosis. The degeneration of the follicle in some way stimulates the oöcyte so that it passes through more or less abnormal maturation stages.

The early prophase is probably passed through very rapidly as no stages were seen of nuclei between the resting stage and the equatorial plate stage. Lams and Doorme ('08) did not observe the stages between the resting nucleus and the first polar spindle in the normal maturation of the egg of the white mouse. Kirk-

("6:3:1"), Hermann's, and Flemming's fluids. Heidenhain's iron hematoxylin, Jenner's blood stain, and Flemming's triple stain were used. Sections were stained over night in Jenner's stain, diluted with three parts of water; this stain was used after fixation in Carnoy's fluid and gave excellent results for some purposes. The cytoplasm of degenerating egg-cells was stained a much deeper pink or red than that of normal egg-cells. The nuclei of follicle cells and phagocytic cells were stained a deep blue. The stain will fade after a time, however.

A modification of the shorter method of Flemming's triple stain was used. Sections of material fixed for two to four hours in Flemming's or Hermann's fluid were bleached in a dilute solution of hydrogen peroxid and after rinsing were placed in a four per cent. solution of ferric alum for four to twelve hours. The sections were then rinsed in distilled water, dipped in the safranin solution a second or two, rinsed again in distilled water, and placed in the gentian violet solution for two to ten or fifteen minutes. Then after rinsing in distilled water the sections were stained in the orange G for ten to sixty seconds. After dehydrating rapidly in absolute alcohol the sections were differentiated in clove oil under the control of the microscope. The clove oil was removed by toluene or xylene and the sections mounted in balsam. By this method the cytoplasm of the oöcytes was stained a yellow-brown, the chromosomes were stained a violet, and the spindle fibers a dark violet—almost purple. The method is rather capricious but when successful, the spindle fibers stand out very distinctly against the yellow-brown cytoplasm of the egg-cell. The method was used principally to bring out the spindle fibers, as the chromosomes are not stained so distinctly or sharply as by the iron hematoxylin method.

The solutions used are as follows:

1. { Safranin, saturated solution in absolute alcohol, 1 part.
   Safranin, saturated solution in distilled water, 1 part.
2. Gentian violet, 1 per cent. solution in distilled water.
3. Orange G, 2 per cent. solution in distilled water.

ham ('08) describes very briefly a few stages of the prophase of the first maturation division in this same form.

Descriptions of the first and second polar spindles in normally maturing oöcytes do not agree. Sobotta ('07) says: "Wenn auch namentlich die Breite individuell etwas wechselt, so beträgt Länge wie Breite des ersten Richtungsspindel der Maus doch stets das Doppelte der Masse des zweiten Richtungsspindel, die Breite ist in der Regel mehr als doppelt so gross." Lams and Doorme, on the other hand, find that the first and second polar spindles are of exactly the same length and diameter. They state: "D'une façon certaine, un ovule à second fuseau ne se distingue d'un ovule à premier fuseau que par la présence, dans le premier cas, du premier globule polaire." Kirkham agrees with Sobotta in saying that the first polar spindle is larger than the second and also in describing the chromosomes as differing in size and shape in the two spindles.

In regard to oöcytes in atretic follicles, Athias says of the spindle, first or second: "Sa forme et ses dimensions sont variables, mais il n'y a pas des caractères qui soient propre au premier ou au second fuseau; la présence concomitante d'un premier globule polaire est, d'après ce que j'ai pu constater dans mes préparations, le seul critérium pour affirmer si l'on est en présence d'un second fuseau de direction." In my own preparations the lengths and diameters of a number of first and second polar spindles in atretic oöcytes were measured. For the first polar spindles, the average length was found to be 24.7 μ, the diameter 12.8 μ, while for the second, the average length was 25.9 μ and the diameter 8.7 μ. Allowing for error in measuring, the spindles are seen to be of about the same length, while the second polar spindles are about two thirds the diameter of the first.

In degenerating oöcytes of the white mouse the first polar spindles are found to be of two general forms: slender, with the achromatic fibers meeting at a point at each end, and thick, "barrel-shaped," with broadly rounded ends. These two kinds are met with in about equal numbers. In both, the chromosomes are arranged with their long axes parallel to that of the spindle. The chromosomes are not arranged around the periphery of the spindle, but are scattered in the plane through the middle of the spindle, at right angles to its length. The chromosomes of the first polar spindle are larger than those of the second and are long with a marked thickening or swelling at the middle. This thickening is at one side of the chromosomes and it is at this point that division takes place. The division is apparently transverse. This account agrees with those of Sobotta and Kirkham for the normal first maturation spindle.

The chromsomes are grouped so closely together and overlap to such an extent that it is difficult to determine their number with accuracy. They apparently vary in number from twelve to twenty-four, the larger numbers being due to a precocious division of some, while others are still undivided.

Descriptions of the appearance of the spindle itself differ. Sobotta, Kirkham, and Lams and Doorme, state that polar radiations or asters are not present in first polar spindles of normally maturing oöcytes, and Athias agrees with them in the case of degenerating egg-cells. Rubaschkin, however, describes in atretic oöcytes of the guinea-pig polar radiations arising from a clear area or centrosphere. Kirkham describes centrosomes consisting of several minute granules at the poles of the spindles. Sobotta states that there are no centrosomes in the first polar spindles of normally maturing oöcytes, and Athias finds none in degenerating egg-cells. In my own preparations radiating fibers are to be seen at the poles of a few first polar spindles; these, however, are not to be considered true asters, but spindle fibers which have broken away from the spindle. This will be discussed more in detail further on. No centrosomes are found in any of these degenerating oöcytes. The spindle fibers are not divided into central spindle fibers and mantle fibers; the fibers with chromosomes attached are intermingled with those which are not connected with chromosomes. The two kinds do not differ in appearance or staining reaction. The spindle itself usually lies at right angles to the radius of the oöcyte, until it swings around to a radial position for the formation of the polar body. (See Figs. 1, 2 and 5.)

The stages in the formation of the first polar body must follow one another rapidly for only a few of these stages were observed. Some of the chromosomes divide earlier than others and consequently the metaphase is not so distinctly marked as in some forms. A few instances of a telophase were seen, in some of which both groups of daughter-chromosomes are in the oöcyte, with no indication as yet of a division of the cytoplasm to form the polar body, while in the others the constricting off of the polar body may be plainly seen. After the first polar body is formed the chromosomes remaining in the oöcyte do not form a resting nucleus but at once enter the second polar spindle.

The second polar spindle, as stated above, is of about the same length as the first, while only two thirds or three fourths as much in diameter. The chromosomes as seen in the equatorial plate stage are short and rod-like and straight or slightly curved. They are not as long or as much curved as Kirkham describes in the second polar spindles of normally maturing oöcytes. The chromosomes are arranged with their long axes parallel to that of the spindle itself and as in the first polar spindle are scattered in a plane at right angles to the length of the spindle. This differs from Kirkham's account of the normal second polar spindle, in which he states that in general the chromosomes lie with their long axes across the spindle. In the spindles of these degenerating egg-cells some of the chromosomes are constricted across the middle in preparation for division, having the appearance of elongated dumb-bells; others have not started to divide and have the typical rod-like form. Others still have already divided and are short and thick, their length only slightly exceeding their diameter. (See Figs. 3 and 4.) This division as well as the first is apparently transverse in the mouse. The chromosomes are crowded together, as in the first polar spindle, making an accurate count difficult; there are from twelve to thirty, owing to the fact that some divide sooner than others.

Descriptions of the appearance of the second polar spindle are as conflicting as those of the first. Sobotta states that in normally maturing oöcytes there are neither centrosomes nor polar radiations. Lams and Doorme describe centrosomes but say that polar radiations are absent; Kirkham states that both centrosomes and polar radiations are present in some cases. In second polar spindles of degenerating oöcytes Athias states that neither centrosomes nor polar radiations are present. In my own preparations radiating fibers are to be seen at the poles of a number of second polar spindles. As in the case of the first polar spindles, however, these are not to be considered true asters, but spindle fibers which have broken away from the spindle, and have assumed a radial position at the ends of the spindles. This will be discussed more in detail later. In general, centrosomes are absent in second polar spindles of atretic oöcytes, but are present in some cases. When they are seen, they consist of a few minute granules which stain deeply and are either in a compact group at the poles of the spindle or are somewhat spread out forming a sort of cap for the spindle. (See Figs. 3, 4 and 6.)

The first polar body is almost always present with the secondary oöcyte in atretic follicles, although in a few instances the spindle has all the characteristics of a second polar spindle while the polar body is not to be seen. In such cases it is possible that the polar body has already degenerated and been absorbed, or as Kirkham suggests for normal oöcytes, it may have been "forced through the zona (pellucida) by the contraction of the latter under the influence of changing osmotic conditions" during fixation. In nearly every instance, however, the polar body is present, lying within the zona pellucida, and somewhat flattened between the oöcyte and the zona. The smaller dimension of the polar body is one half or two thirds of the larger, while the larger diameter itself is a fifth to a third that of the oöcyte. In a few cases the polar body contains a spindle more or less deranged and abnormal (see Fig. 3) but usually the chromosomes are scattered through it irregularly. They may be grouped in a few large irregular masses of chromatin or there may be a number of smaller chromosomes of abnormal size and shape. In a few cases a resting nucleus may be seen in the polar body (Fig. 10). The second polar spindle is usually found in the oöcyte near the polar body, at right angles to the radius of the egg-cell. Rarely it may be seen in the other side of the oöcyte, and a few spindles have been seen in a radial position. A few instances were observed where the oöcyte contained two spindles; this is probably due to the fact that the egg-cell had two nuclei to start with.

Up to this point oöcytes in follicles undergoing follicular atresia have passed through the same stages, with some differences in detail, as normally maturing egg-cells. The later stages however are different. The next step in degeneration is the breaking down of the spindles. The usual course is for the oöcytes to form the second polar spindles which then break down; but if degeneration has proceeded a little more rapidly, this fate may overtake the first polar spindles before the polar body is formed.

As stated above, in the first polar spindle as well as in the second, the achromatic fibers are all intermingled, those with chromosomes attached and those without, and do not differ in appearance or staining reaction. The fibers with no chromosomes attached to them break across at their middle and the free ends move out in the cytoplasm. As the polar ends remain attached to the poles of the spindles, the formation of "asters" is brought about. Stages are seen (Figs. 5 and 6) in which the breaking or splitting off of the fibers is taking place; some of the fibers have just broken across, in the figures, and others have already assumed a radial position, giving the appearance of "asters." The achromatic fibers with chromosomes attached next break or split off, and as their free ends move out into the cytoplasm, they draw after them the attached chromosomes (see Fig. 7). In this way more fibers are added to the "asters" and chromosomes are seen connected with the ends of some of the fibers. This splitting off of the achromatic fibers explains the fact that some spindles have radiating fibers or "asters" at their poles, while in others they are absent. The oöcytes containing spindles without radiating fibers have not advanced so far in degeneration that the spindle fibers have begun to split off. The result of this splitting off of the fibers and the consequent breaking down of the spindles is that the chromosomes are scattered in all directions in the cytoplasm at each pole of the spindle, while still connected with the poles by the spindle fibers.

The cause of the breaking down of the spindles is to be found in the degeneration of the oöcyte. That this degeneration has proceeded to quite an extent is shown by the presence in the cytoplasm of fat-granules and crystalloid bodies, and by the fact that the cytoplasm stains much more deeply with acid stains such as eosin and orange G than does the cytoplasm of normal oöcytes. The spindle fibers share in this degeneration and show it first by breaking across and splitting off from the spindle. Rubaschkin states that as the fibers split off, the poles of the spindles approach each other and finally come to lie so close together that it is difficult or impossible to distinguish one from the other. While this account of the breaking down of the spindles agrees essentially with that of Rubaschkin for the guinea-pig, nothing resembling the approach of the poles of the spindles was observed in the mouse.

The achromatic fibers soon disappear and the chromosomes thus left free in the cytoplasm of the oöcyte begin to form nuclei. Each chromosome forms a small vesicle which has the appearance of a vacuole with the chromatin material massed at one side (Fig. 13). In some instances the chromatin is arranged in small granules around the outer part of the vesicle (Fig. 8). As this process goes on, the vesicles near enough together coalesce to form larger ones (Figs. 8, 9, 11), while those isolated in the cytoplasm remain separate. In this way a varying number of nuclei are formed, of different sizes. A nucleus formed by the combining of a number of chromosomes is larger than one formed from a single chromosome. The final number of nuclei thus formed may be from two to twelve, depending on how the chromosomes were scattered in the oöcyte. These nuclei are transformed into resting nuclei of more or less normal appearance.

The nucleo-cytoplasmic relationship, already interfered with by the degenerative changes in the egg-cell, is further disturbed by this formation of a number of small nuclei. The size-relationship, as well as the morphological, physiological, and chemical, relationship, is clearly affected. Apparently there is an effort, even in the degenerating oöcyte, to restore as far as possible this size-relationship, and this effort is expressed by a breaking up of the cytoplasm into smaller parts around the various nuclei. A part of the cytoplasm may surround several of these small nuclei when these are close together, or may enclose only one, when they are isolated. It occasionally happens that a bit of the cytoplasm may fail to contain even one of these nuclei, when part of the egg-cell was without any nuclei as a result of an incomplete scattering of the chromosomes. The result of this breaking up of the oöcyte is that there are formed a number of small "cells," some with several nuclei, some with one, and some with none, so that the oöcyte has the appearance of a "morula." The fact that some "cells" have nuclei and others have not, is due to the uneven distribution of the chromosomes in the cytoplasm of the oöcyte when the spindle breaks down. In general, the "cells" containing large nuclei, or a group of nuclei, are larger than those with one nucleus or none. However, a definite or effective control over this fragmentation is apparently lacking.

Several authors, Newman among others, have described cells in this "morula" stage which have spindles in them, and state that these are cleavage spindles and that therefore this is a case of parthenogenetic cleavage. It is more probable, however that in such cases the cell containing the spindle is the first polar body, which, as noted above, occasionally forms a spindle, and which may in rare instances divide. In the white mouse no spindle was found in any of the cells of this "morula" stage.

In the cells of the "morula," and sometimes in the oöcyte before it has fragmented, are frequently found crystalloid bodies the nature and origin of which are unknown. Possibly they are a product of the degenerative changes in the egg-cell. Fat granules are found in the oöcytes in increasing numbers as degeneration goes on.

In a few instances the oöcyte is found to have formed two cells of nearly equal size, each containing a nucleus. Van der Stricht describes such cases in the bat and states that each cell may divide again, and each of the four cells thus formed may also divide. The formation of two such equal cells may be explained on the grounds that the scattered chromosomes were arranged in two groups and formed two nuclei; the oöcyte then broke up into two fragments of equal size. Such an egg-cell is shown in Fig. 10, with the first polar body also present; but the two nuclei are not equal in size, nor normal in appearance. In fact, one is apparently little more than a vacuole.

The fate of the "morula" may be briefly described. The zona pellucida usually persists as a thick transparent membrane for some time after the oöcyte itself has completely degenerated and disappeared, although in a few instances it is absorbed early. Phagocytic (?) cells make their way into the oöcyte through the zona pellucida, and are probably to be regarded as follicle cells from the degenerating follicle. These cells are usually seen in the outer border of the oöcyte, or just outside it, lining the inner surface of the zona pellucida, sometimes as early in the course of degeneration as the spindle stage. There are not many of these cells in a single oöcyte, not more than eight or ten and frequently no more than three or four. They may be imbedded in the cytoplasm of the egg-cell and in the "morula" stage are frequently seen in between the separate cells. One case is illustrated (Fig. 14) showing one of these extra-ovular cells just after it has entered the oöcyte, still retaining its connection with other follicle cells outside the egg-cell by means of a protoplasmic process extending through the zona pellucida. This same cell is also shown to be connected with one or two other similar cells within the zona by other protoplasmic processes, forming a sort of syncytial net-work or mesh-work in among the fragments of the degenerating egg-cell.

The cytoplasm of these cells is usually rather scanty and sometimes they look like bare nuclei imbedded in the cytoplasm of the oöcyte (Figs. 8 and 9). They are not, however, to be confused with the nuclei of the oöcyte formed by the breaking down of the spindle, for they react differently to the stains used and have a different structure. They are finely granular and these granules are stained an intense black by iron hematoxylin and deep blue by Jenner's stain.

It may be through the action of these cells that the fragments of the oöcyte are gradually absorbed and disappear, for later on the zona pellucida is seen, shrunken and distorted, with a few of these cells in a remnant of the egg-cell. In still later stages, these cells are seen alone inside the zona, and this condition may persist for some time (Fig. 16). Eventually the zona pellucida and these cells all disappear and by this time the follicle itself has usually completely degenerated.

Thus it is seen that the oöcytes in atretic follicles in the ovary of white mice not yet sexually mature undergo a series of changes which in the early stages at least resemble maturation. That these changes are in some way correlated with the atresia is shown by the fact that all the oöcytes exhibiting these phenomena are found in atretic follicles, and egg-cells of normal appearance are seen in the follicles not yet overtaken by atresia. The degeneration of the follicle stimulates the oöcyte to pass through a process which at first resembles maturation but which later results in a breaking up of the egg-cell into fragments, some with nuclei and some without. In the light of the evidence here presented, this process can not be considered parthenogenetic cleavage for no mitotic figures other than those of a more or less abnormal maturation were seen; and if this were true parthenogenetic cleavage it would be expected that some stages of mitosis would be observed. The absence of mitotic figures other than more or less normal polar spindles, the breaking down of these spindles, the scattering of the chromosomes, the formation of nuclei from these chromosomes, and the consequent breaking up of the egg-cell into small parts, with or without nuclei, show rather conclusively that in the white mouse, not yet sexually mature, the process is one of degenerative fragmentation.

Summary.

The spindles seen in oöcytes in follicles undergoing atresia folliculi are maturation spindles, more or less abnormal, and not cleavage spindles.

By the splitting off of the achromatic fibers and the consequent breaking down of these spindles the chromosomes are scattered through the cytoplasm of the oöcyte and form a number of nuclei.

The nucleo-cytoplasmic relationship, disturbed by the degenerative changes in follicle and egg-cell, causes the oöcyte to break up into fragments, some with one or more nuclei and some with none. These fragments are gradually absorbed, probably through the action of phagocytic cells of follicular origin, and disappear.

The process is one of degenerative fragmentation and not parthenogenetic cleavage.

BIBLIOGRAPHY.

Athias, M.

'09 Les phénomènes de division de l'ovule dans les follicules de De Graaf en voie d'atrésie chez le Lérot. Anat. Anz., Bd. 34, pp. 1–23.

Bonnet, R.

'99 Giebt es bei Wirbeltieren Parthenogenesis? Erg. Anat. u. Ent., Bd. 9, pp. 820–870.

Flemming, W.

'85 Über die Bildung von Richtungsfiguren in Säugerthiereiern beim Untergang Graaf'scher Follikel. Arch. f. Anat. u. Phys., 1885, p. 221–244.

Kirkham, W. B.

'08 Maturation of the egg of the white mouse. Trans. Conn. Acad., Vol. 13, pp. 65–87.

Lams, H. et Doorme, J.

'08 Nouvelles recherches sur la maturation et la fécondation de l'oeuf des mammifères. Arch. de Biol., T. 23, pp. 259–365.

Newman, H. H.

'13 Parthenogenetic cleavage of the armadillo ovum. Biol. Bull., Vol. 25, pp. 54–78.

Rubaschkin, W.

'06 Über die Veränderungen der Eier in den zugrunde gehenden Graafschen Follikeln. Anat. Hefte., Bd. 32, H. 97, pp. 255–278.

Sobotta, J.

'07 Die Bildung der Richtungskörper bei der Maus. Anat. Hefte., Bd. 35, Bd. 35, H. 106, pp. 493–552.

Spuler, A.

'01 Über die Teilungserscheinungen der Eizellen in degenerierenden Follikeln des Säugerovariums. Anat. Hefte., Bd. 16, H. 51, pp. 85–114.

Van der Stricht, O.

'01 L'atrésie ovulaire et l'atrésie folliculaire du follicule de De Graaf dans l'ovaire de Chauve-souris. Verhandl. d. anat. Gesellsch. 15. Versamml. Bonn., 1901, p. 108–121. (Anat. Anz. Ergänz. z. Bd. 19, pp. 108–121.)

EXPLANATION OF PLATES.

All the figures are camera-lucida drawings made from the actual preparations. All the drawings except Figs. 3, 7, and 14, were made by Miss Cora J. Whitman.

PLATE I.

FIG. 1. Primary öocyte, containing a first polar spindle. The chromosomes are longer and more slender than usual in first polar spindles. The zona pelludica is seen surrounding the egg-cell (zp.). × 670.

FIG. 2. Primary oöcyte containing a first polar spindle, of the "barrel-shaped" type. The chromosomes are of the type usual for this spindle. The zona pellucida has disappeared. × 670.

FIG. 3. Secondary oöcyte, with second polar spindle and polar body which also contains a spindle more or less deranged. Some of the chromosomes of the egg spindle have been omitted from the drawing in order to show more clearly the characteristic shape of the chromosomes of the second polar spindle. × 670.

FIG. 4. Secondary oöcyte, with second polar spindle and polar body. Centrosomes are seen at each end of the spindle. × 916.

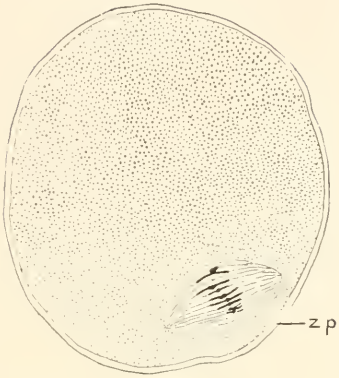

Fig 1

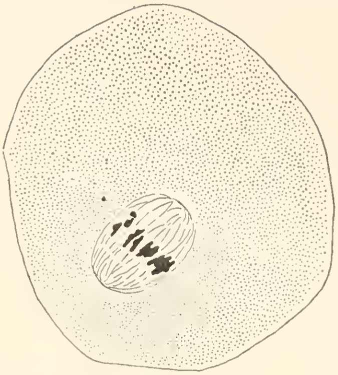

Fig 2

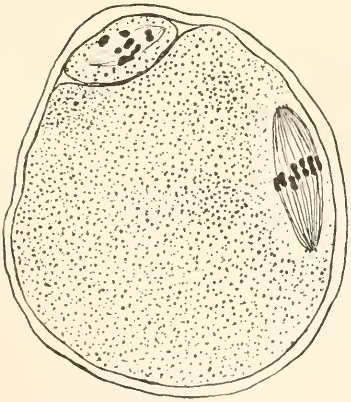

Fig 3

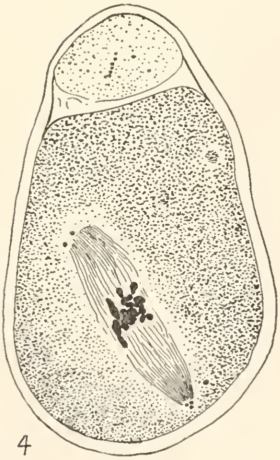

Fig 4

H. M KINGERY.

PLATE II.

FIG. 5. First polar spindle alone, showing achromatic fibers splitting off, forming "asters." × 916.

FIG. 6. Second polar spindle alone, showing well-defined "asters," formed by fibers which have split off from the spindle. × 916.

FIG. 7. Primary oöcyte showing spindle seen obliquely from one end, which has broken down. The scattering of the chromosomes is partially accomplished. ×670.

FIG. 8. Egg-cell with nuclei formed from scattered chromosomes. The bodies at the end of the oöcyte (*x*) are probably cells formed by the division of the polar body. A crystalloid body (*c*) and an extra-ovular cell (*p*) are also shown. ×670.

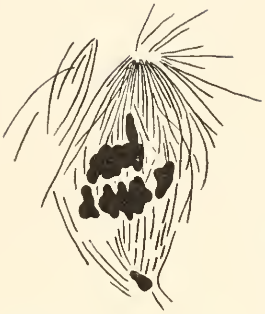

Fig 5

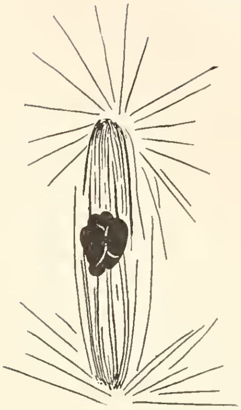

Fig 6

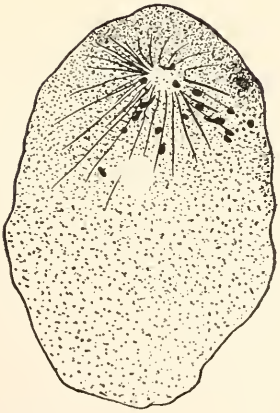

Fig. 7.

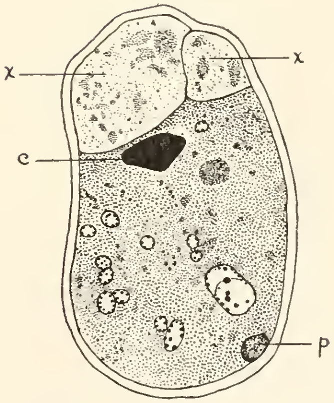

Fig 8.

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PLATE III.

FIG. 9. Egg-cell containing four nuclei; these, from their size, have been formed by the coalescence of a number of smaller vesicles. Two extra-ovular cells (*p*) are shown. × 670.

FIG. 10. Oöcyte divided into two more or less equal parts, with polar body also present (*pb*). The nuclei are not alike, one being apparently only a hollow vesicle. × 670.

FIG. 11. Egg-cell in several fragments, one of which contains five nuclei. The zona pellucida is broken in two places and extra-ovular cells are present between the fragments. × 670.

FIG. 12. "Morula" stage, some fragments with nuclei and others without. × 670.

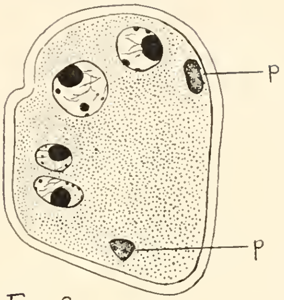

Fig 9

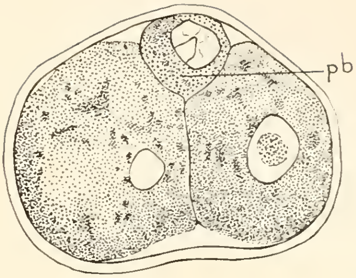

Fig 10

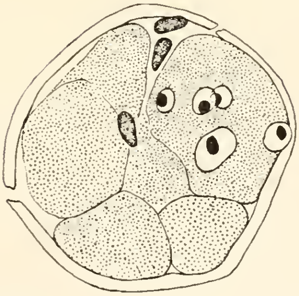

Fig 11

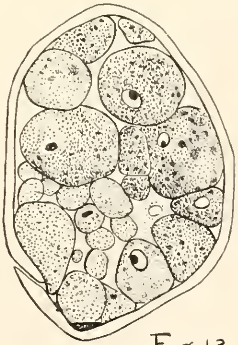

Fig 12

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PLATE IV.

FIG. 13. Egg-cell showing small nuclei formed from the scattered chromosomes. The chromatin material is massed at one side of each of the vesicles. × 670.

FIG. 14. Egg-cell containing extra-ovular cells connected by protoplasmic processes. One is still connected with the follicle cells outside by a process extending through the zona pellucida. × 670.

FIGS. 15 AND 16. Final stages in degeneration. Zona pelludica with remnant of oöcyte and a few extra-ovular cells inside. × 670.

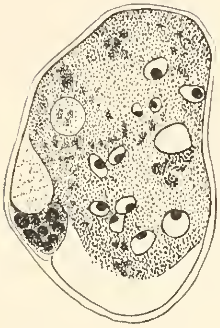


Fig. 13

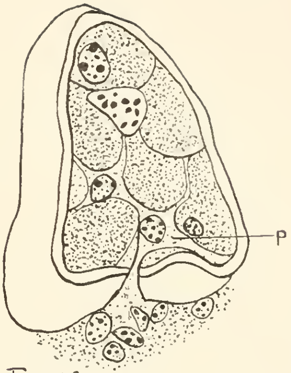


Fig 14

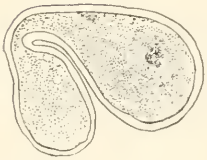


Fig 15

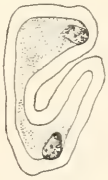


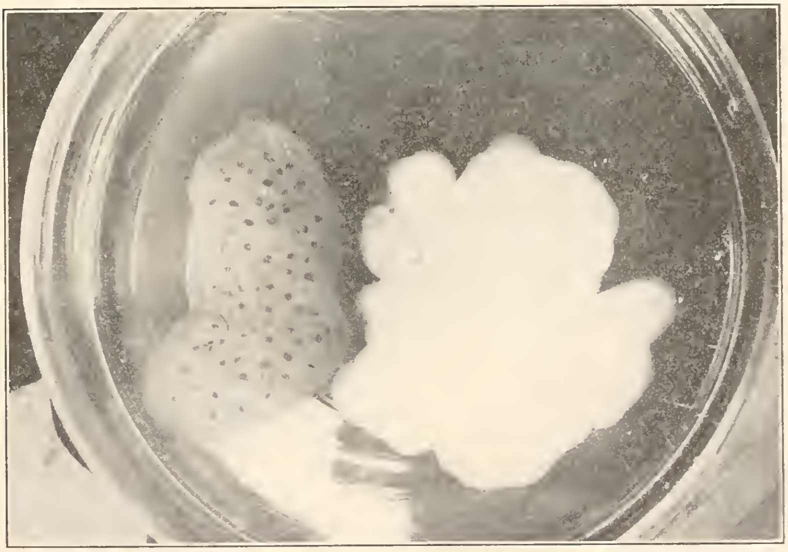
Fig 16

H. M. KINGERY

A MILKY WHITE AMPHIBIAN EGG JELL.

ARTHUR M. BANTA AND ROSS AIKEN GORTNER.

While collecting *Ambystoma punctatum* eggs in April, 1914, the writers found a freshly-laid bunch which had jell of milky whiteness instead of being transparent. The white clutch of eggs was conspicuous among the others even though the jell had as yet swelled very little and the bunch was quite small. The eggs were still undivided. They had the normal amount of pigment. The jell was so clouded that at first no eggs could be seen in the mass without breaking into the jell. The jell imbibed water and the egg bunch soon assumed the normal proportions for the clutch of this species. The photograph shows the relative opacity of the white bunch and a normal bunch of eggs of the same age. Only a few eggs were visible in the opaque bunch when the photograph was taken while one could readily see the bottom of the dish through the jell of the normal bunch. The individual outer and inner egg membranes in the white bunch were of normal transparency. This is indicated indistinctly



in the photograph by the somewhat more transparent spots, in which were eggs, near the edges of the white bunch.

When just ready to hatch the embryos with their egg membranes were removed from the jell, care being taken to remove all traces of eggs which had died during development. The water was carefully drained off and the jell was dried in a porcelain dish over a water bath. The jell from a normal egg bunch of the same stage of development was treated in a similar manner. After drying on the water bath the jell was further desiccated in a vacuum desiccator over sulfuric acid.

233 grams of normal egg jell yielded 0.7855 grams or 0.337 per cent. of dry material.

294 grams of the opaque jell yielded 1.060 grams or 0.361 per cent. of dry material.

It thus appears that the jell which normally surrounds these eggs contains about 99.6 per cent. of water and yet this jell is fairly tough and resistant and admirably suited to supporting the eggs in such a position as to permit ready diffusion of oxygen and carbon dioxide and to protect the eggs from mechanical injury.

The two jells when desiccated were indistinguishable. A portion of each was again placed in water and in a short time they had imbibed enough water to resume their former appearance, the one becoming milky white and the other transparent. It may perhaps be germane to add that the milky appearance could not be ascribed to bacteria inasmuch as the egg mass was found shortly after being deposited and also because there was no evidence of bacterial decomposition up to the time the drying was begun.

Thinking that perhaps the milky color was produced by an admixture of albumen with the mucin (which composes the normal egg jell) nitrogen determinations were made of both abnormal and normal egg jells.

The nitrogen was determined by Kjeldahl's method.

Normal egg jell.—0.2060 gram gave 12.3 c.c. 0.1 normal NH_4OH; 0.3564 gram gave 21.1 c.c. 0.1 normal NH_4OH, indicating 8.36 per cent. and 8.29 per cent. respectively or an average of 8.32 per cent. of nitrogen in the normal egg jell.

Opaque egg jell.—0.2764 gram gave 18.3 c.c. 0.1 normal NH_4OH; 0.3540 gram gave 23.0 c.c. 0.1 normal NH_4OH, indicating 9.27 per cent. and 9.09 per cent. respectively or an average of 9.18 per cent. of nitrogen in the opaque egg jell.

Almost no chemical work has been done with the amphibian egg jells aside from the observation that the nitrogen content is low due to the presence of a carbohydrate nucleus. Of course the above results do not give the true nitrogen content of the egg jell for they should have been corrected for ash content, but this was not possible due to lack of material. The figures do show, however, that the jells differ chemically as well as in appearance, and that the difference in nitrogen content is in the same direction and of almost precisely the same amount that it would be if the opaque appearance were produced by an admixture of albumen (nitrogen = 15 per cent.–16 per cent.) with the normally occurring mucins.

Station for Experimental Evolution,
August 17, 1914.

THE RELATION OF THE BODY TEMPERATURE OF THE EARTHWORM TO THAT OF ITS ENVIRONMENT.

CHARLES G. ROGERS AND ELSIE M. LEWIS.[1]

In a paper by one of us upon the "Temperature Coefficient of the Rate of Heart Beat in Certain Living Animals"[2] the assumption was made that the temperature of the living animal (worm or fish-embryo) under observation corresponded very closely to that of the water surrounding it. The same assumption has been made by other workers in this field, *e. g.*, Snyder[3] in his work upon the isolated heart of the Pacific terrapin assumed that the temperature of the more or less bulky heart muscle of the terrapin was conditioned by the temperature of the solution in which it was placed. Robertson[4] also in his work upon *Ceriodaphnia* assumed that the temperature of the water definitely represented the temperature of the tissues with which he was particularly concerned. Even more recently Loeb and Ewald[5] make use of the same assumption.

There are to be found in the literature of physiology statements concerning the body temperatures of the so-called cold-blooded animals, and an examination of the data offered reveals the fact that the various investigators who have taken the trouble to make any examination of the actual conditions find that the temperatures of the animals studied vary considerably from the temperatures of the surroundings. It is also true that many of the observations recorded were made by means of mercurial thermometers, though some were made by thermocouples with galvanometers. In reading the statements of the different investigators one is left with the feeling that with the more improved appliances of the present day one ought to be able to make observations which would be more satisfactory than those published. The following table summarizes the results of a number of investigators, and is compiled from data quoted by Milne-Edwards.[1]

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[1] From the Department of Zoology, Oberlin College.

[2] Rogers, Charles G., "Studies upon the Temperature Coefficient of the Rate of Heart Beat in Certain Living Animals," *American Journal of Physiology*, 1911, Vol. 28, No. II., pages 81–93.

[3] Snyder, C. D., "On the Influence of Temperature Upon Cardiac Contraction and Its Relation to Influence of Temperature upon Chemical Reaction Velocity," University of California Publications, Physiology, Vol. 2, No. 15, 1905.

[4] Robertson, T. B., "Note on the Influence of Temperature Upon the Rate of the Heart Beat in a Crustacean (*Ceriodaphnia*)," BIOLOGICAL BULLETIN, 1906, Vol. X., No. 5, pp. 242–248.

[5] Loeb, Jacques and Ewald, W. F., "Die Frequenz der Herztätigkeit als eindeutige Funktion der Temperatur," *Biochemische Zeitschrift*, 1913, Bd. 58, H 3, 177–185.

Animal.					Authority.
Fishes	less than 1°C.	above	surrounding	water	Milne-Edwards
Frog	0°.32–2°.44	"	"	"	Czermak
Frog	0°.0 –0°.575	"	"	"	Becquerel
Frog	0°.04	"	"	"	Dutrochet
Toad	0°.2	"	"	"	"
Frog	0°.7 –0°.3	"	"	"	Dumeril
Proteus	1°.25	"	"	"	Rudolphi
Proteus	2°.6 –5°.6	"	"	"	Czermak
Crayfish	6°.0	"	"	"	Rudolphi
Maia sq.	0°.3 –0°.9	"	"	"	Valentin
Limax	0°.33–0°.50	"	"	"	Spallanzani
Snail	2°.0	"	"	"	Hunter
Snail	1°.1	"	"	"	Martine
Snail	0°.9	"	"	"	Becquerel
Snail	1°.5 –2°.0	"	"	"	Schnetzler
Aplysia	0°.1 –0°.8	"	"	"	Valentin
Octopus	0°.2 –0°.6	"	"	"	"
Eledone	0°.9	"	"	"	"
Annelids	0°.56–0°.85	"	"	"	Hunter
Lumbricus	1°.11–1°.39	"	"	"	"
Holothuria	0°.2 –0°.6	"	"	"	Valentin
Ophiurian	0°.3	"	"	"	"
Asterias ru.	0°.6	"	"	"	"
Sea Urchin	0°.4 –0°.5	"	"	"	"
Medusa Pelagia	0°.2 –1°.0	"	"	"	"
Medusa Cassiopea	0°.3	"	"	"	"
Actinians	0°.2 –0°.5	"	"	"	"

In the table it will be noted that the temperatures determined for the different animals show, for the most part, rather small variations from that of the surrounding water. In a few cases the variation is quite considerable, and appears to make desirable a reëxamination of the facts. This is especially true in view of the fact that certain scientific friends have raised question as to the validity of the assumption upon which the temperature coefficient work was based. It is with an idea of attempting to answer any questions as to the propriety of assuming that the temperature of the earthworm is represented by the temperature of the surroundings, that the present investigation is here reported.

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[1] Milne-Edwards, "Leçons sur la Physiologie et L'Anatomie Comparée de L'Homme et des Animaux," T. VIII., Paris, 1863.

METHODS.

A method of measuring differences of temperature by means of the electromotive force developed when the junctions of wires of different metals of a common circuit are not at the same temperature was described by Nobili and Melloni[1] about 1830. Since that time galvanometers have been made more sensitive, and it has also been made possible to obtain pure metallic wires of small diameter, and of small heat capacity. The authors named were the first to apply this method of temperature measurement to living animals, and now that the methods of use have been somewhat improved the same method has been employed for the measurement of the amount of heat given off in a given contraction of a frog muscle.[2] The method can be made accurate enough to measure differences as small as 1/150° C. For the purpose of the investigation here reported it did not seem necessary to make measurements as small as those recorded by Hill, so the number of junctions of the wires was not at all increased. The thermo-couples used consisted of No. 32 copper and No. 32 constantan wires joined together as shown in Fig. 1. In some of the couples the wires were simply twisted together and in others the junction was made secure by a small drop of solder. We were not able to determine that for our purpose the soldered junctions were any more efficient than those not soldered. The junction used within the body of the worm was mounted within a slender glass tube in such a way as to have the two wires of the couple thoroughly insulated from each other except at the junction point (Fig. 2). This was accomplished by placing one of the wires in a finely drawn glass tube, allowing the end of the wire to extend slightly below the end of the tube, where it was twisted together with the other wire of the couple. The small glass tube with the two wires was then placed inside another glass tube of a diameter just sufficient to receive them easily. The glass of the outer tube was then sealed over the junction of the wires, and the whole bent into a convenient form for handling. The upper open ends of the tubes were sealed with wax to prevent the access of any water and the apparatus was ready for use.

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[1] Nobili et Melloni, "Recherches sur plusiers phenomenes calorifiques enterprises au moyen du thermo-multiplcaiteur," *Ann. de chimie et de physique*, 1831, T. XLVIII., p. 208.

[2] Hill, A. V., "The Energy Degraded in the Recovery Process of Stimulated Muscles," *Journal of Physiology*, 1913, Vol. 46, pp. 28–80.

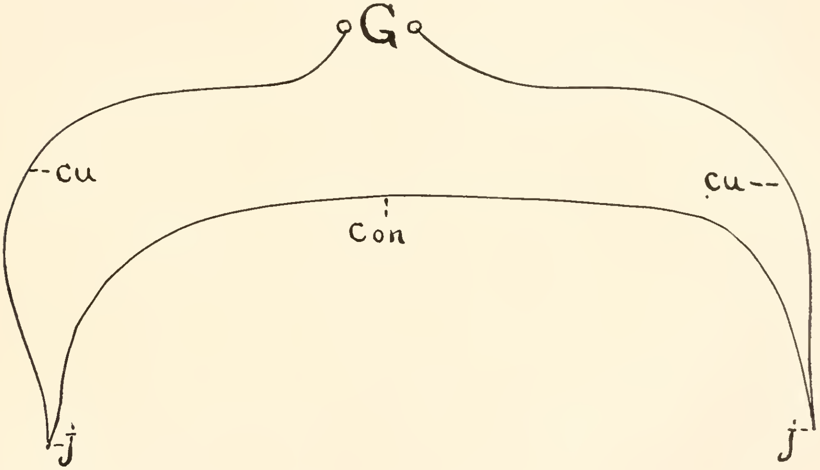

FIG. 1. Diagrammatic scheme of thermocouple. *G* = galvanometer; *cu* = copper wire; *con* = constantan wire; *j* = junction.

The galvanometer used in this work was one of the D'Arsonval type made by Gaertner. It was provided with a dead beat coil, and was so heavily damped that it was found desirable to allow two or three minutes for the galvanometer mirror to come to rest when making observations. It was found that with the scale at about 1 meter distance 1° C. difference in the temperature of the two junctions of the thermo-couple was represented by a shifting of the reading of the galvanometer scale by about 16 millimeters. The actual amount of shift varied somewhat from day to day from this value, and was re-determined for each day's work.

The technic of determining the temperature of the interior of the worm was very simple. It consisted in placing the glass-covered junction of the thermocouple in the mouth of the worm, and carefully pushing it down through the œsophagus, crop and gizzard until it came to lie in the stomach intestine. The other junction of the couple was placed in water of a known temperature, and the two end wires of the thermocouple were attached to the galvanometer. The temperature of the water in which the free junction of the thermocouple was placed was determined by a Beckman thermometer which had been set to a definite reading by the side of a certified standard thermometer. The temperature of the water was determined to 1/100° C., and was noted for every observation so that in case of any variation in the temperature of the water we should be able to make the necessary correction in the results. The temperature of the worm was shown by the amount of the deviation of the galvanometer reading, from the zero reading, divided by the number of millimeters representing 1° C., and adding this amount to the known temperature of the water. In this way we were able to make readings which could be accepted as accurate to within 0.03° C., which for our purpose seemed to be sufficient.

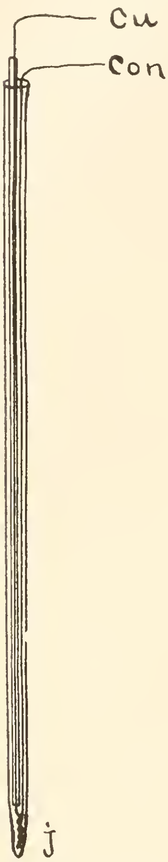

FIG. 2. Sketch of thermo-electric clinical thermometer for use with the earth-worm. *cu* = copper wire; *con* = constantan wire; *j* = junction.

The following table gives data derived from three experiments to show how closely and how rapidly the temperature of the worm becomes adjusted to the temperature of the water in which it is immersed. We have not thought it necessary to multiply examples as all the facts observed are in perfect harmony with those offered. In the experiments here cited both the worm and the free junction of the couple were placed in the same bath. The difference between the zero and in-circuit readings of the galvanometer would then indicate the difference in temperature between the temperature of the water and the interior temperature of the worm if any such difference exists. After each reading the worm and free junction of the couple were placed in the water of the temperature indicated for the next following reading.

Time.	Temp. of Water in Which Worms Were Placed.	Zero Reading of Galvanometer.	Galvanometer Reading Couple in Circuit.	Difference in Millimeters.	Temperature of Worm.
A. 9.40	11.40° C.	345	345	000	11.40° C.
10.10	21.30	345	345	000	21.30
B. 9.00	21.24	403			
9.17	21.24	403	402	001	21.30
9.25	10.00	403	403	000	10.00
9.35	21.20				
9.40	21.20	408	408	000	21.20
9.55	13.00	408			
10.00	13.00	408	408	000	13.00
C. 10.30	21.20	403	404	001	21.14
10.40	17.00	403	404	001	17.94
10.45	21.20	403	404	001	21.14

The above data are representative of a large number of observations made during an investigation upon the effect of temperature changes upon the rate of contraction of the dorsal blood vessel of the earthworm, *Lumbricus agricola*, and indicate very clearly that the animal under investigation adapts itself with remarkable quickness and closeness to the temperature of its environment. In fact we think it may safely be said that the worm will adapt itself to a change of at least ten degrees Centigrade within two minutes, to an accuracy of 0.05° C. This fact makes it possible then to use the temperature of the water surrounding the animal as an indicator of the temperature of the animal, in the case of the earthworm, for experiments upon the temperature coefficient of heart action, and assures us that the worm need not be subjected to a bath of a given temperature for any great length of time in order to get an accurate result. It is very likely true that the same principle will be found to hold good for other animals of a similar kind and habit, certainly for marine worms, fish embryos, small crustacea, etc. It is the purpose of the authors to continue the investigation upon other forms in order to determine to what extent we may be at liberty to assume the temperature of the surrounding fluid to be an indicator of the temperature of the tissues.

BIBLIOGRAPHY.

Hill, A. V.

'13 The Energy Degraded in the Recovery Processes of Stimulated Muscles. Journal of Physiology, Vol. 46, pp. 28–80.

Loeb, Jacques and Ewald, W. F.

'13 Die Frequenze der Herztätigkeit als eindeutige Funktion der Temperatur. Biochemisches Zeitschrift, Bd. 58, H. 3, 177–185.

Milne-Edwards.

'63 Lecons sur la Physiologie et L'Anatomie Comparee de L'Homme et des Animaux, T. VIII., Paris.

Nobili et Melloni.

'31 Recherches sur plusiers phenomenes calorifiques enterprises au moyen du thermo-multiplicateur. Ann. de Chimie et de Physique, T. 48, p. 208.

Robertson, T. B.

'06 Note on the Influence of Temperature Upon the Rate of Heart Beat in a Crustacean (*Ceriodaphnia*). BIOLOGICAL BULLITIN, Vol. X, p. 242.

Rogers, Charles G.

'11 Studies upon the Temperature Coefficients of the Rate of Heart Beat in Certain Living Animals. American Journal of Physiology, 1911, Vol. 28, pp. 81–93.

Snyder, Charles D.

'05 On the Influence of Temperature upon Cardiac Contraction and its Relation to Influence of Temperature upon Chemical Reaction Velocity. University of California Publications, Vol. 2, No. 15, pp. 125–146.

THE TEMPERATURE COEFFICIENT OF THE RATE OF CONTRACTION OF THE DORSAL BLOOD-VESSEL OF THE EARTHWORM.

CHARLES G. ROGERS AND ELSIE M. LEWIS.[1]

Certain unpublished criticisms of a previous paper of the senior author[2] have led us to make a further investigation of the effects of changes of temperature upon the rate of contraction of the dorsal blood vessel of the earthworm. The criticism offered against the previous work was that no evidence was presented to show that the temperature of the forms studied, worms and fish-embryos, was the same as that of the water in which they were immersed. The force of the criticism is recognized, and we are now able to present the results of an investigation in which the temperature of the worm studied was determined by means of a delicate clinical thermometer inserted in the long, tubular, alimentary canal of the worm.

We are now publishing under another title[3] an account of the work in which it is shown that the temperature of the surrounding water does furnish an excellent indicator of the inner body temperature of the earthworm, when this animal is immersed in water for experimental purposes. Inasmuch as this is true we have no doubt that the previous work upon *Nereis*, *Tubifex*, and the embryos of *Fundulus* and the toad-fish will bear the same sort of inspection.

We will not at this time take up any discussion of the literature of the subject. The papers of Snyder, Robertson, Loeb and Ewald and others are available for examination. The formula employed for the computation of the temperature coefficients is the one used by Snyder in all his work, and concerning which he has presented some matters of historical interest,[1]

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[1] From the Department of Zoology, Oberlin College.

[2] Rogers, Charles G., "Studies Upon the Temperature Coefficient of the Rate of Heart Beat in Certain Living Animals," *American Journal of Physiology*, 1911, Vol. XXVII., pp. 81–93.

[3] Rogers, Charles G., and Lewis, Elsie M., "The Relation of the Body Temperature of the Earthworm to that of its Surroundings," BIOLOGICAL BULLETIN, 1914, Vol. XXVII., pp. 261–267.

$$Q_0 = \left(\frac{K_1}{K_0}\right)^{\frac{10}{T_1 - T_0}}.$$

The material used in this study was the large earthworm, *Lumbricus agricola*. This form presents two desirable features for this work; 1st, the animals are not by nature restricted to any definite or narrow limits of temperature at which their normal physiological processes take place; and 2d, it is well adapted structurally for the work in that it is easily possible to place a delicate temperature measuring apparatus in the alimentary canal, and it is also easy to see the contractions of the dorsal blood vessel through the more or less transparent body wall. This last fact is of special importance as it makes it practicable to leave the animal undisturbed in its constant temperature bath, thus obviating any disturbance of the vascular contractions through nervous action due to stimulation from without.

METHODS.

As a preliminary to the actual temperature work a number of worms were subjected to immersion, for varying periods, in water to ascertain what harmful effects might result. Without going into detail as to this work it may be stated that the worms are able to withstand immersion in tap water for a sufficient time to allow all the experimentation needed for the temperature studies, without showing any harmful effects. In fact certain worms have been immersed for as long a period as two weeks without showing any injurious effects.

The temperatures of the worms were regulated, then, by placing them in baths of water, the temperature of which was controlled by placing the dishes in thermostats having practically constant temperatures, in refrigerators cooled by ice, or in the running water of the laboratory, which was found to have a very constant temperature.

The temperatures of the worms were determined by means of delicate clinical thermometers, in the form of thermo-couples, which could be inserted into the mouths of the worms and pushed on down into the stomach intestine. These thermo-couples were made of No. 32 copper and No. 32 constantan wires. Any difference in the temperature of the two junctions of the couple sets up an electromotive-force proportional in its strength to the amount of the temperature difference, and which can be accurately measured by means of a delicate galvanometer. In this particular work it was found that 1° C. was represented by a shift in the reading of the galvanometer scale of about 16 mm. As it was practicable to read to half millimeters it will be seen that a temperature difference of 0.03° could be determined. For a somewhat more detailed account of the method of temperature measurements the reader is referred to another paper.[1]

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[1] Snyder, C. D., "On an Interpolation Formula Used in Calculating Temperature Coefficients for Velocity of Vital Activities, Together with a Note on the Velocity of Nerve Conduction in Man," Science, N.S., Vol. XXXIV., No. 874, p. 415.

The worm having been subjected to a given temperature for a sufficient length of time to have become completely adjusted to the new condition, the temperature of the worm was noted, and the length of time required for a definite number of beats of the dorsal blood-vessel, usually 25, was taken by means of a stop-watch reading to fifths of a second. The worm was then changed to another bath at a different temperature, allowed to remain long enough to become thoroughly adjusted to the new condition, and another reading of the rate of contraction of the dorsal blood vessel made. From the data thus obtained the temperature coefficient of the rate of contraction was calculated by means of the formula referred to above.

It may be said that all possible precautions were taken to avoid serious errors in the work. Temperatures were determined by making the readings of the galvanometer against a certified thermometer calibrated to tenths of a degree C. The temperatures of the various baths were kept as uniformly constant as possible and the exact temperature taken each time a count was made. Occasionally one is in doubt as to whether a contraction of the dorsal blood vessel has actually taken place. In such a case the reading was thrown out and another made so as to make sure of the fact. The temperature of the room was kept as constant as possible in order to avoid any changes of resistance in the copper wires and in the galvanometer which might tend to disturb the results. Manipulation of the worms was reduced to a minimum in order that nervous effects might not be introduced to invalidate the temperature effects. Reading of the rates of contraction of the dorsal blood vessel before and after the insertion of the glass covered junction indicated that the mere insertion of the instrument in the alimentary canal made no difference with the rate.

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[1] Rogers, Charles G., and Lewis, Elsie M., *l. c.*

TABLE I.

No. of Worm.	K_1	K_0	T_1	T_0	Q_{10}
3	19.35	13.74	21.43°	11.60°	1.406
5	15.00	9.80	18.00°	11.50°	1.925
6	20.35	10.90	18.00°	11.50°	2.593
7	21.10	13.80	16.04°	11.50°	2.548
8	15.72	8.92	16.04°	11.50°	2.096
9	22.00	9.93	16.04°	11.50°	5.766
10	16.93	10.60	16.04°	11.50°	2.805
11	17.75	11.29	16.04°	11.50°	2.642
12	14.79	11.76	16.04°	11.50°	1.656
14	27.00	19.40	26.40°	11.70°	1.272
16	26.90	14.92	26.40°	11.60°	1.485
17	15.27	11.13	26.50°	11.60°	1.236
18	19.70	15.33	26.50°	11.60°	1.176
19	29.30	12.50	27.13°	12.55°	1.793
21	24.15	13.50	27.12°	12.55°	1.280
24	33.60	24.00	27.00°	16.36°	1.372
26	37.80	26.70	26.60°	24.60°	5.754
27	32.56	19.35	20.00°	9.50°	2.044
28	35.27	24.55	20.00°	9.50°	1.413
29	21.39	8.85	27.50°	14.00°	1.621
30	28.20	12.13	27.10°	14.00°	1.899
31	20.40	13.18	27.70°	14.00°	1.365
32	27.48	11.75	27.30°	14.04°	1.898
33	21.50	12.66	26.50°	14.02°	1.525
34	26.55	14.05	25.60°	14.03°	1.733
35	22.00	12.95	25.08°	13.88°	1.605
36	24.86	12.63	25.08°	13.92°	1.838
37	16.97	8.95	22.54°	13.95°	2.109
38	20.40	11.58	22.46°	13.96°	1.947
39	22.43	11.86	21.86°	13.94°	2.236
40	21.59	9.81	21.43°	13.95°	2.871
41	26.80	11.16	22.90°	13.72°	2.603
42	21.97	11.10	22.95°	13.74°	2.099
43	23.33	11.94	22.71°	13.80°	2.121
44	25.00	11.23	23.18°	13.73°	2.388
Average value of temperature coefficient for all specimens.......					2.173

The preceding table, Table I., gives the data derived from the actual experiments and also the values of the temperature coefficient calculated from the data. In the tables the letters

K_1 and K_0 indicate the rates in contractions per minute at the temperatures T_1 and T_0 respectively, in degrees Centigrade.

If we arrange the data of Table I. so as to show the relation existing between the higher and lower temperature ranges and the value of Q_{10} we have evidence that for the lower temperatures there is a higher coefficient than for the higher ranges. This fact is not so clearly shown in the case of the earthworm as in some of the forms already studied, probably for the reason that it is very difficult to count the beats of the dorsal blood vessel of the earthworm at temperatures below 8° C. The movements of the walls are so feeble and so slow that one is not sure when a contraction has taken place.

TABLE II.

T_1.	T_0.	Number of Worms.	Average Q_{10}.
About 27° to	about 14°	4	1.671
“ 27° “	“ 12°	2	1.536
“ 26° “	“ 11°	4	1.292
“ 25° “	“ 13°	3	1.725
“ 22° “	“ 13°	6	2.199
“ 21° “	“ 13°	2	2.533
“ 20° “	“ 9°	2	1.728
“ 16° “	“ 11°	6	2.919

In Table II. we have such an arrangement of the data as suggested in the preceding paragraph. It will be noted that while in general the coefficients for the lower ranges are higher than those for the higher ranges of temperature, there are very marked exceptions to the rule. The only explanation we have to offer at this time for the rather marked variation from what we should expect, if the beat of the dorsal blood vessel of the earthworm is subject to the same laws as the beats of various hearts already studied, is that we have here to deal with a series of nervous effects which must be in some way eliminated in order to avoid complications. Up to the present time we have not found any means of avoiding these nervous effects in the worm, though in fish embryos where the rate of heart beat was studied before the nervous connections were established it was found that one could predict with some degree of certainty what the rate of heart contraction should be at any stated temperature.

That the rate of beat of the dorsal blood vessel of the earthworm is to some extent under the control of the nervous system will be shown in another publication from this laboratory.

It is to be noted also that the temperature coefficient of the rate of contraction of the dorsal blood vessel of the earthworm is, for the temperatures at which the worm would naturally live, of the same general magnitude as those of chemical reactions, and the average for the whole series, 2.173, also falls within the limits usually set for the temperature coefficients of such reactions. We have no reason, as yet, to assign for the much reduced coefficients for the higher temperature ranges.

BIBLIOGRAPHY.

Loeb, Jacques and Ewald, W. F.

'13 Die Frequenz der Herztätigkeit als eindeutige Funktion der Temperatur. Biochemisches Zeitschrift, Bd. 58, pp. 177–185.

Rogers, Charles G.

'11 Studies upon the Temperature Coefficient of the Rate of Heart Beat of Certain Living Animals. American Journal of Physiology, Vol. 28, pp. 81–93.

Snyder, Charles D.

On the Influence of Temperature upon Cardiac Contraction and its Relation to Influence of Temperature upon Chemical Reaction Velocity. University of California Publications, Physiology, Vol. 2, pp. 125–146.

Snyder, Charles D.

On an Interpolation Formula used in Calculating Temperature Coefficients for the Velocity of Vital Activities, together with a note on Nerve Conduction. Science, N.S., Vol. 34, No. 874, p. 415.

Snyder, Charles D.

The Influence of Temperature upon the Rate of Heart Beat in the Light of the Law for Chemical Reaction Velocity. American Journal of Physiology, 1906, Vol. 17, No. 4.

Snyder, Charles D.

The Temperature Coefficient of the Velocity of Nerve Conduction. American Journal of Physiology, 1908, Vol. 22, No. 1.

Snyder, Charles D.

A Comparative study of the Temperature Coefficients of the Velocities of Various Physiological Actions. American Journal of Physiology, 1908, Vol. 22, No. 3.

Woodruff, L. L. and Baitsell, G. A.

The Temperature Coefficient of the Rate of Reproduction of Paramoecium aurelia. American Journal of Physiology, 1911, Vol. 29, No. 2.

AUDITORY POWERS OF THE CATOCALA MOTHS; AN EXPERIMENTAL FIELD STUDY.[1]

C. H. TURNER AND E. SCHWARZ.

HISTORICAL RESUMÉ.

Near the close of the nineteenth century, Romanes ('91) wrote: "Among insects organs of hearing certainly occur, at least in some, although the experiments of Sir John Lubbock appear to show that ants are deaf. The evidence that some insects are able to hear is not only morphological, but also physiological, because it is only on the supposition that they do that the fact of stridulation and other sexual sounds being made by certain insects can be explained, and Brunelli found that when he separated a female grasshopper from the male by a distance of several meters, the male began to stridulate in order to inform her of his position, upon which the female approached him. I have myself published observations proving the occurrence of a sense of hearing among the Lepidoptera."

The tone of three fourths of the above paragraph is characteristic of practically all of the early works upon the auditory powers of insects. Those men were convinced that insects hear; not because they had experimentally demonstrated it, but for morphological reasons, and because many kinds of insects can produce sounds. They believed that an insect would not be endowed with the power of producing sounds unless the other members of the species could hear. At first in the Orthoptera and later in other groups of insects, peculiar organs were found; consisting essentially of vibratory hairs attached to certain cells that seem to be sensory in nature. In some cases these hairs are in cavities and in others they are not. Such was the nature of the work of Siebold ('44), Leydig ('55), Henson ('66), Lee ('83, '85), Graber ('75, '82), Weinland ('91), Adelung ('92) and others. As late as 1905 Radl expressed the following thought. No matter how often in recent years doubt has arisen as to the ability of insects to hear, it has been largely maintained that they possess an auditory sense, and for the following two reasons: (1) the ability of many insects to produce sounds as a part of their normal behavior; (2) the possession by insects of organs which structurally seem fitted to act as receptors of sound waves.

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[1] For the identification of the species and for the experimental work on *C. unijuga*, E. Schwarz alone is responsible; the field work was performed jointly; for the planning of the work, for the historical resume and for the method of treatment, C. H. Turner is solely responsible.

Students interested in the morphological method of investigating this question will find the paper by Radl ('05) intensely interesting. After epitomizing the work by Lee ('83, '85), by Graber ('82), and by Weinland ('91), he states that, on a priori grounds, he doubted the assumptions of Graber; but that certain experiments had convinced him that insects have a crude auditory sense. In support of his contention, he offers the following reasons.

1. Graber is inaccurate when he claims that the chordotonal organs are located rigidly between two immovable parts of the body; for the proximal end is attached to an indifferent part of the body, while the distal end is in close proximity to one or more muscles.

2. The chordotonal organ cannot function like a string attuned to a certain pitch; because it expands and contracts.

3. All of the chordotonal organs examined are attached at each end to a chitinous framework and the nerve penetrates from the side.

4. The chordotonal organs resemble somewhat those muscles which occur especially in the limbs of the Arthropoda—muscles which terminate in long tendons.

5. No chordotonal organ is found in either the Myriapoda or the Arachnida.

6. Chordotonal organs are found in some insects for which a sense of hearing could have no significance. They are well developed in caterpillars; even in those of the Tortricidæ, which spend the entire larvel period inside of some fruit. They are also well developed in the internal parasites of certain insects.

7. All attempts to determine experimentally that insects react to pure and simple tones have yielded negative results: however, it is comparatively easy to evoke responses of insects to shrill noises, such as the voice of a cricket or the screech produced by

drawing a file across the edge of an iron or a glass plate. This is not a tactile reaction.

8. There is no evidence that noise, as such, causes the orientation of insects. The sounds produced by insects are more an outburst of inner feeling than an attempt to entice the female by the male.

9. The outcome of the whole matter is that there is an auditory sense in insects; but, it is on a much lower plane of development than that of the vertebrates. Its anatomical and physiological antecedents are to be found, not in the tactile organs and contact activities; but, anatomically in sense organs which register muscle activities and physiologically in general sensation (Gemeingefuhlen). The auditory sense of insects is a highly refined muscular sense.

Although the work of the early investigators was largely, often entirely, morphological, it must not be concluded that no experimental work has been done on the auditory powers of insects and their near kin. Such experiments have been performed in several groups of insects; but the results are inharmonious. Buttel-Reepen ('00) and De Fraviere believe that bees can hear. Buttel-Reepen's statement is based upon his observation that bees respond in a definite manner to the sounds of their own kind.

Huber ('10) and Forel ('03) interpret their experiments to mean that ants cannot hear. Lubbock's experiments likewise yielded negative results; yet, in spite of this, he was unwilling to admit that ants, wasps and bees cannot hear. Weld ('99) thinks he has experimentally demonstrated that ants can hear. Fielde and Parker ('04) interpret their experiments to mean that ants do not respond to sound vibrations as such. C. H. Turner ('07) is equally positive that his experiments demonstrate that ants can hear. At one time Wheeler believed, not only that ants can hear; but that they communicate by means of sounds; but, after the appearance of the paper by Fielde and Parker, he ('10) asserts that there is not sufficient evidence to warrant the assumption that ants can hear.

E. A. Andrews ('11) is convinced that termites hear. He writes: "In a community suspended from the ceiling by a copper wire and represented by many thousands on a moist block of artificial stone which they got to from the nest by means of a long stick as bridge, it was first observed by Mr. Middleton that the noise of thunder and of blasting rocks was followed by a quick and very remarkable departure of almost all of the termites towards the nest. The blocks of stone weighed some sixteen pounds each and rested in a large pan of water on a firm wall of stone, so that it seemed likely that the concussion of the air came to the termites directly and not as a tremor of the stones they were clustered on. The same precipitous flight of the multitude of termites from these stones to the nest along the bridge was brought about by dropping a board upon the concrete floor with a loud crash. Even the clapping of hands, which probably shook the stone foundation but imperceptibly, served to drive the termites back to the nest. . . . Attempts to influence the termites by blowing horns of various pitches near them failed though considerable disturbance of the air was produced."

Montgomery ('10), after reviewing all that had been written on the auditory powers of spiders, concludes that spiders are deaf.

The above is not an exhaustive discussion of the published research work on the auditory powers of insects other than the Lepidoptera; yet, we trust it is sufficient to show the inharmoniousness of the results of different investigators.

To the best of our knowledge, the first published results of experiments upon the auditory powers of butterflies and moths is an article by Romanes ('76) which appeared about thirty-six years ago. The portion referring to the Lepidoptera is so short that we quote it in full. "It seems worth while to add a few words with respect to the sense of hearing in insects. So far as I am aware, the occurrence of such a sense in this class has never been actually proved. Although on *a priori* grounds there can scarcely be any doubt concerning the fact of some insects being able to hear; seeing that in so many species stridulation and other sounds are made during the season of courtship. In the case of moths, however, I believe that sounds are never emitted—except, of course, the death's head moth.[1] It therefore becomes interesting to observe that an auditory sense is certainly present in these insects. Several kinds of moths have the habit of gently, though very rapidly, vibrating their wings, while they themselves are at rest on a flower or other surface. If, while this vibrating movement of the wings is going on, the observer makes a sudden shrill note with a violin, or fife, etc., the vibrating movement immediately ceases, and sometimes the whole body of the insect gives a sudden start. These marked indications of hearing I found invariably to follow a note with a high pitch, but not a note with a low one."

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[1] Romanes was mistaken when he asserted that the death's head moth is the only Lepidopteran than produces sounds; for the literature contains records of

Heinrich ('09) remarks that collectors using a net to capture *Limenitis populi* and *Catocala fraxini* have observed that the insects often take flight before a collector is near enough to capture them. Accordingly to him no conclusive evidence has been published on the sense of hearing of insects, especially of the Lepidoptera. He noticed a *Laurentia suffumata* alight in a concert pavilion and remain quietly while the brass band played three selections, one of which was Wagner's Götterdammerung. He also noticed that certain Lepidoptera were more easily approached at twilight than when the sun was shining brightly, and he could not understand why this should be true if they were warned by a sense of hearing. He is convinced that, in all of these cases, it is vision, not audition, that warns butterflies and moths of the approach of man.

Hamann ('09) was led to investigate this subject by the remarks of collectors that butterflies and moths undoubtedly hear. One collector remarked that the noise caused by removing the cork from the cyanide bottle often caused these insects to fly. To this Haman replies that since the net is usually placed beneath the insect before the cork is removed it is probable that the sight of the net caused the flight. To test the matter, the following experiments were performed by him. (1) He approached a tree in such a manner as to be invisible to an *Apatura iris* L. resting thereon, and struck the tree with the bamboo handle of his

several species of Lepidoptera that emit sounds. Indeed, scarcely had his article appeared before several of his contemporaries published, in *Nature*, protests in which were cited several examples of sound-producing Lepidoptera. Recently Omensetter ('12) and Stephan ('12) have described several sound-producing forms of butterflies and moths.

collecting net. To this the insect made no response; but, as soon as the net approached it took flight. (2) a repetition of the experiment with *Vanessa* yielded similar results. (3) He noticed that certain Catocalas, which were not disturbed by the noise of a passing automobile, flew upon the approach of man. These experiments convinced him that butterflies and moths cannot hear.

Deegener's work ('09) is morphological. Between the thorax and the abdomen, on the ventral side of the body of all species of Nocturidæ, there is a depression containing chitinous structures and hairs which are connected with what seem to be sensory cells. A careful examination of this organ in *Pseudophia lunaris* convinced Deegan that it is probably an auditory organ.

Rothke ('09) confined a *Limenitis artemis* in a cage which he placed on the top of a pedestal two feet high. The sides and back of this cage were constructed of wood; but the front was covered with wire fly netting. At nine P.M., while the front of the cage was illuminated by means of a kerosene lamp, Rothke stepped to one side and made a slight noise. To this the insect made no response. The investigator then tapped rapidly and sharply upon the floor with a leather slipper. Although the jar was not sufficient to shake the stand upon which the cage rested, and although the investigator could not be seen by the insect, yet it slowly raised its wings until they met above its back and then lowered them again. Several repetitions of this experiment yielded identical results. During the intervals between the experiments the creature remained immobile. After the moth had been quiet for one quarter of an hour, Rothke rapped upon the table with a tumbler. Immediately the insect flapped its wings. About midday he discovered a specimen of *Catocala unijuga* resting quietly, about six feet from the ground, upon a pine tree one and a half feet in diameter. He picked up a stone about fifteen centimeters in diameter and threw it against the tree-trunk. Although the moth could not see the stone and although the blow was too slight to jar such a large tree, yet the moth flew away. Rothke is convinced that butterflies and moths can hear.

Observations made upon *Catocala pacta* L. convinced Richter

('09) that the crackling of twigs under his feet and even the slight noise made by removing the cork from his collecting bottle disturbed this moth. In another article, the same investigator ('10) has made a comparative study of the auditory powers of day-flying and night-flying Lepidoptera. He investigated the day-flyers; *Apatura* sp., *Vanessa* sp., *Limenitis populi* L., and *Sat. alcyone* Schiff. He found that species of *Apatura* and of *Vanessa* made no responses to sound so long as no visible object disturbed them. During a severe storm, he noticed a number of *Sat. alcyone* perching on a limb. Neither whistling, nor the clapping of hands, nor shaking of the limb had any effect on them: but, as soon as the hand of the collector approached them, they flew. A *Vanessa antiopa*, resting on a telegraph pole, was not disturbed by the shrill whistle and the rumbling noise of a passing train. In studying *Catocala fraxini* L., a night-flying moth, he noticed that it made no responses to the noises made by wagons, automobiles and the bells of a ferry; but that it responded readily to slight, high-pitched, sounds. He argues that the failure of this moth to respond to the sounds made by wagons and such things is because such sounds have no life significance for the moth. On the other hand, the ready response to the other sounds mentioned is due to their similarity to sounds made by field-mice, bats and owls—sounds which for the moth have a pronounced life significance. Partly influenced by the knowledge that Geegener had discovered chordotonal organs in the Noctuidæ and more so by the observations just described, Richter is convinced that day-flying Lepidoptera are warned by visual and night-flying forms by auditory stimuli.

For years Rober ('10) has raised *Acerontia atropos* L. from pupae. Late one evening a female emerged and, before her wings were fully dried, a male emerged in the cage. In order to separate the two, the female was removed to a cage in the bottom of which there was a crack as wide as one's finger. These cages were three meters apart. In the morning the female, which had escaped from confinement, was found perched on the cage containing the male. A person who slept in the room with these two moths asserts that, for a long time that evening, those moths emitted sounds. Rober concludes that these were love calls and that the moths mutually heard. In face of the well known fact that the sense of smell is well developed in butterflies and moths, the evidence just cited does not appear to be conclusive.

Is it possible for anyone to read the above historical resume and not be convinced that there is need for much exact experimentation upon the auditory powers of insects? Evidently the last word has not been spoken.

Description and Discussion of Experiments.

Reading Stephan's ('12) recent articles on sound producing butterflies and moths induced in us the same thought that influenced the opinions of many of the early investigators; namely, animals that produce sounds as a part of their normal behavior can probably hear. We decided to make some crucial experiments. The Catocala moths were selected for the following reasons: (1) one of us is so well acquainted with the taxonomy of the group that it is easy to identify species afield; (2) the habit these moths have of resting during the day on some tree trunk and, when disturbed, flying to a nearby tree trunk renders them ideal material for field experiments.

Preliminary Observations.

Observations afield taught us that there are certain sounds to which these moths do not respond. A favorite haunt of the Catocala moths of this vicinity is a small stretch of wood through which a railroad passes. Moths resting on trees near the tract are not disturbed by the whistle, rumble and roar of passing trains. Near that same place there is a pleasure garden in which the sounds of a noisy piano are often heard. No responses to the strains of the piano were noticed.

These observations are in harmony with those of Heinrich ('09), Hamann ('09) and Richter ('09, '10).

Indoor Experiments on Catocala unijuga.

Three specimens freshly hatched from pupae were the subjects of these experiments. Each was kept in a separate room. Three times a day, for four days, the auditory powers of these moths was tested by whistling in a high key. Occasionally the moth was shielded from the draft of air caused by whistling;

TABLE I.

Catocala unijuga. SPECIMEN NUMBER 1.

Number of the Experiment.	Date.	Whistled, the Insect Shielded from the Air Currents.	Whistled, the Insect Not Shielded from the Air Currents.	Number of Times it Was Necessary to Whistle Before Response Was Received.
1	June 5, 1913, 10:00 A.M....	*N*		
2	" " " ...		*F*	1
3	" " " ...		*F*	1
4	" " " ...	*F*		4
5	" " " ...	*Q*		5
6	June 5, 1913, 10:30 A.M....		*F*	2
7	" " " ...	*Q*		6
8	" " " ...	*F*		3
9	" " " ...	*Q*		8
10	June 5, 1913, 6 P.M........	*F*		4
11	" " "	*Q*		3
12	" " "	*F*		6
13	" " "	*Q*		10
14	June 6, 1913, 9:00 A.M.....	*N*		
15	" " "	*Q*		4
16	" " "	*F*		2
17	" " "	*Q*		5
18	June 6, 1913, 11.00 A.M....	*F*		4
19	" " " ...	*Q*		10
20	" " " ...	*F*		4
21	" " " ...	*Q*		15
22	June 6, 1913, 6:00 P.M.....	*F*		8
23	" " "	*Q*		15
24	" " "	*F*		6
25	" " "	*Q*		19
26	June 7, 1913, 9:00 A.M.....	*F*		4
27	" " "	*Q*		10
28	" " "	*F*		4
29	" " "	*Q*		6
30	June 7, 1913, 10:00 A.M....	*F*		3
31	" " " ...	*Q*		8
32	" " " ...	*F*		3
33	" " " ...	*Q*		15
34	June 7, 1913, 7:00 P.M.....	*F*		6
35	" " "	*Q*		5
36	" " "	*F*		4
37	" " "	*Q*		6
38	June 8, 1913, 10:00 A.M....	*F*		2
39	" " " ...	*Q*		10
40	" " " ...	N_4		
41	" " " ...	*Q*		6
42	June 8, 1913, 10:30 A.M....	*N*		
43	" " " ...	*Q*		6
44	" " " ...	N_2		
45	" " " ...	*Q*		10
46	" " " ...	*F*		2
47	June 8, 1913, 6:00 P.M.....	*F*		4
48	" " "	*Q*		3
49	" " "	*F*		6
50	" " "	*Q*		10
51	" " "	*F*		13

EXPLANATION OF TABLES I–III.

N indicates no response; *F* means that the moth flew; *Q* indicates that the insect waved its wings up and down or made some quivering movement.

TABLE II.

Catocala unijuga. SPECIMEN 2.

Number of the Experiment.	Date.	Whistled, the Insect Shielded from the Air Currents.	Whistled, the Insect Not Shielded from the Air Currents.	Number of Times it Was Necessary to Whistle Before the Moth Responded.
1	June 6, 1913, 9:00 A.M.	*Q*		6
2	" " "		*F*	2
3	" " "	*F*		5
4	" " "	*Q*		4
5	June 6, 1913, 10:00 A.M. . . .		*F*	1
6	" " " . . .	*F*		4
7	" " " . . .	*Q*		3
8	" " " . . .	*F*		2
9	June 6, 1913, 6:00 P.M.	*F*		1
10	" " "	*Q*		3
11	" " "	*F*		2
12	" " "	*Q*		6
13	June 7, 1913, 10:00 A.M. . . .	*F*		2
14	" " " . . .		*F*	
15	" " " . . .	*F*		4
16	" " " . . .	*Q*		5
17	June 7, 1913, 11:00 A.M. . . .	*F*		2
18	" " " . . .	*QF*		4
19	" " " . . .	*F*		4
20	" " " . . .	*Q*		4
21	June 7, 1913, 6:00 P.M.	*F*		3
22	" " "	*Q*		3
23	" " "	*F*		2
24	" " "	*Q*		8
25	June 8, 1913, 9:00 A.M.	*N*		
26	" " "		*F*	1
27	" " "	*Q*		3
28	" " "	*F*		8
29	June 8, 1913, 11:00 A.M. . . .	*F*		4
30	" " " . . .	*Q*		6
31	" " " . . .	*F*		4
32	June 8, 1913, 7:00 P.M.	*F*		10
33	" " "	*Q*		6
34	" " "	*F*		2
35	" " "	*Q*		6
36	June 9, 1913, 9:00 A.M.		*F*	2
37	" " "	*Q*		10
38	" " "	*F*		2
39	" " "	*Q*		10
40	June 9, 1913, 11:00 A.M. . . .		*F*	2
41	" " " . . .	*Q*		9
42	" " " . . .	*F*		1
43	" " " . . .	*Q*		11
44	June 9, 1913, 7:00 P.M.	*Q*		6
45	" " "	*F*		1
46	" " "	*Q*		6
47	" " "	*F*		10

TABLE III.

Catocala unijuga. SPECIMEN NUMBER 3.

Number of the Experiment.	Date.	Whistled, the Insect Shielded from the Air Currents.	Whistled, the Insects Not Shielded from the Air Currents.	Number of Times it Was Necessary to Whistle Before the Response.
1	June 7, 1913, 9:00 A.M.....	*N*		
2	“ “ “		*F*	1
3	“ “ “	*Q*		3
4	“ “ “	*F*		2
5	“ “ “	*Q*		10
6	June 7, 1913, 10:00 A.M....	*F*		2
7	“ “ “ ...	*Q*		6
8	“ “ “ ...	*F*		2
9	“ “ “ ...	*Q*		10
10	June 7, 1913, 7:00 P.M.....	*F*		2
11	“ “ “	*Q*		10
12	“ “ “	*F*		4
13	“ “ “	*Q*		15
14	June 8, 1913, 9:00 A.M.....	*F*		3
15	“ “ “	*Q*		6
16	“ “ “	*F*		4
17	“ “ “	*Q*		10
18	June 8, 1913, 10:00 A.M....	*N*		
19	“ “ “ ...	*Q*		10
20	“ “ “ ...	*F*		6
21	“ “ “ ...	*Q*		5
22	June 8, 1913, 10:00 P.M....	*F*		6
23	“ “ “ ...	*Q*		4
24	“ “ “ ...	*F*		4
25	“ “ “ ...	*Q*		10
26	June 9, 1913, 9:00 A.M.....	*F*		4
27	“ “ “	*Q*		3
28	“ “ “	*F*		6
29	“ “ “	*Q*		10
30	June 9, 1913, 11:00 A.M....	*F*		6
31	“ “ “ ...	*Q*		10
32	“ “ “ ...	*F*		5
33	“ “ “ ...	*Q*		8
34	June 9, 1913, 6:00 P.M.....	*F*		10
35	“ “ “	*Q*		2
36	“ “ “	*F*		4
37	“ “ “	*Q*		6
38	June 10, 1913, 9:00 A.M....	*Q*		1
39	“ “ “ ...	*N*		
40	“ “ “ ...	*Q*		6
41	“ “ “ ...	*F*		2
42	“ “ “ ...	*Q*		7
43	June 10, 1913, 11:00 A.M...	*N*		
44	“ “ “ ...	*Q*		3
45	“ “ “ ...	*F*		1
46	“ “ “ ...	*Q*		11
47	June 10, 1913, 6:00 P.M....	*N*		
48	“ “ “ ...	*F*		2
49	“ “ “ ...	*Q*		6
50	“ “ “ ...	*F*		1
51	“ “ “ ...	*Q*		10

but, in some cases, the air current was allowed to strike the moth. The results of those experiments are recorded in tables I.–III.

On the twelfth of June the three specimens, which, up to that time, had been confined in separate rooms, were marked and placed in the same room. At nine A.M. that day, on the first sound of the whistle, they all flew, one after another, as though the flight of the first had evoked the flight of the others. At ten o'clock, the whistling caused two to fly and the other to quiver. The one that quivered was about ten feet away. On whistling again all flew. At six P.M. the whistling caused all to fly.

To our way of thinking this series of experiments is very instructive. That each of these three specimens responded to the whistle on the twelfth of June is unequivocal; that they usually responded to the whistle by either flying or by quivering is also evident; but, it is equally certain that two out of the three specimens did not respond to the whistle at all the first time it was sounded and that the third specimen responded in a feeble manner. When the moths did not respond to the blowing of the whistle at the beginning of the experiment, the current of air produced by whistling was allowed to strike the moth; immediately it flew, and thereafter it would usually fly when the whistle was sounded. There were some exceptions to this; but, in the main it was true. This seems a hint that the moth responds to sounds that have a life significance.

Field Experiments.

These experiments were conducted in a small stretch of woods at Meramec Highlands, near St. Louis, Mo. Previous experience had taught us that these insects would not respond to loud sounds of low pitch. For that reason we used as the sound producing instrument a Galton whistle set to give a high shrill note. One of us would stand where the moth could be observed; but far enough away not to disturb it. Experience had taught us what would be a safe distance. The other, whistle in hand, would approach the tree on the opposite side to that on which the moth was resting. When this experimenter was near to the tree the whistle was held at about the level of the moth and sounded one or more times. In such a position it was absolutely impossible for the moth to see either the whistle or the experimenter. The whistle was usually 180° from the moth; but occasionally it was placed ten to fifteen degrees away, but out of sight of the moth. In a few rare cases, for a special purpose, the whistle was blown in the presence of the moth. Whenever that was done it is indicated in the tables. The results of these experiments are recorded in Tables IV.–XI. The tables are self explanatory.

TABLE IV.

RESPONSES OF *Catocala flebilis* TO SOUND.

Number of the Experiment.	Number of Times the Whistle Was Sounded.	Pitch of the Whistle.	Kind of Response.
1	1	e^5	Flew.
2	1		Flew.
3	1		Flew.
4	1		Flew.
5	5		Flew.
6	3		Flew.
7	1		Flew.
8	1		Flew.
9	1		Flew.
10	1		Flew.
11	1		Moved its antenna, but did not fly.
12	1		Flew.

TABLE V.

RESPONSES OF *Catocala habilis* TO SOUND.

Number of the Experiment.	Number of Times the Whistle Was Sounded.	Pitch of the Whistle.	Kind of Response.
1	1	a^4	Flew.
2	1		Flew.
3	1		Flew.
4	1		Flew.
5	1		Flew.
6	1		Flew.

In the experiments recorded in this table (Table V.) the Galton whistle was held three feet from the tree on which the moth was resting.

TABLE VI.

RESPONSES OF *Catocala neogama* TO SOUND.

Number of the Experiment.	Number of Times the Whistle Was Sounded.	Pitch of the Whistle.	Kind of Response.
1	1	e^5	No response.
2	1		No response.
3	1	a^4	Flew (saw the whistle).
4	1	a^4	Flew.
5	1		Flew.

TABLE VII.

RESPONSES OF *Catocala piatrix* TO SOUND.

Number of the Experiment.	Number of Times the Whistle Was Sounded.	Pitch of the Whistle.	Kind of Response.
1	2	b^4	No response observed.
2	1		Flew.
3	1		Flew.
4	1		Moved its wings up and down
5	1		Ditto.
6	1		Flew.
7	1		No response observed.
8	1		Moved its wings up and down
9	1		Ditto.
10	1		Flew.
11	14		No response observed.
12	1		Moved its wing up and down.

TABLE VIII.

RESPONSES OF *Catocala relicta* var. *luctuosa* TO SOUND.

Number of the Experiment.	Number of Times the Whistle Was Sounded.	Pitch of the Whistle.	Kind of Response.
1	1	e^5	Made quivering movements with its wings.
2	1		Ditto.
3	1		Ditto.
4	1		Ditto.
5	1		Ditto.
6	1		Ditto.
7	1		Ditto.
8	1	a^4	Flew (It saw the whistle).
9	1		Whole body quivered.
10	1		Ditto
11	1		Ditto.
12	1		Ditto.
13	1		Ditto.
14	1		Ditto.
15	1		Ditto.
16	1		Ditto.
17	1		Ditto.
18	1		Ditto.
19	1		Ditto.
20	1		Ditto.
21	1		Ditto.
22	1		Ditto.
23	1		Moved antennæ gradually forward and then flew.
24	1		Body quivered.
25–35			Ditto.
36	1		Flew.
37	1		Whole body quivered.
38–43	1		Ditto.
44	1		Moved its antennæ four times and then flew.

TABLE IX.

RESPONSES OF *Catocala robinsoni* TO SOUND.

Number of the Experiment.	Number of Times the Whistle Was Sounded.	Pitch of the Whistle.	Kind of Response.
1	1		Flew.
2	1		Flew.
3	1		Flew.
4	1		Flew.

TABLE X.

RESPONSES OF *Catocala vidua* TO SOUND.

Number of the Experiment.	Number of Times the Whistle Was Sounded.	Pitch of the Whistle.	Kind of Response.
1	1	a^4	No response observed.
2	1		Flew.
3	1		Flew.
4	1		Flew.
5	1		Flew.
6	1		No response observed.
7	1		Ditto.
8	1		Ditto.
9	1		Ditto.
10	1		Ditto.
11	1		Ditto.
12	1		Flew (It saw the whistle).
13	1		No response observed.
14	1		Flew.
15	1		Flew.
16	1		No response observed.
17	1		Flew.
18	1		No response observed.
19	1		Flew.
20	4		Flew.

TABLE XI.

RESPONSES OF *Catocala vidua*, SPECIMEN NUMBER 2, TO SOUND.

Number of the Experiment	Number of Times the Whistle Was Sounded.	Pitch of the Whistle.	Kind of Response
1	1	a^4	Moved its antennæ.
2	1		Flew.
3	1		Flew.
4	19		Flew, but not until after the nineteenth whistle.
5	1		Flew.
6	4		No response noticed.
7	4		No response noted.
8	1		Flew (Saw the whistle).

In June, 1914, we made an attempt to see if, in the field, moths could be trained to respond to sounds to which they do not normally respond. We knew that this can be done in the laboratory. Our experience the year before had informed us that most Catocalæ do not respond to sounds of a low pitch. We selected an organ pipe giving 256 vibrations per second. This was sounded several times and if the moth did not respond it was sounded again and simultaneously one of us touched the moth with a brush. We then followed the moth to its next resting place and sounded it again, and if necessary, repeated it over and over.

For these experiments we used; *C. amica*, *C. epione*, *C. neogamma*, *C. ilia*, and *C. innubens*. With *innubens* and *epione* all results were negative. We found two specimens of *ilia* which responded to the sound of the pipe before they had been touched in any manner and one that did not so respond. This response from *ilia* was unexpected, but it militated against using it for these experiments. We succeeded in inducing one specimen of *amica* to respond to the pitch; but failed completely with two others. We experimented with five specimens of *C. neogama*, all males. We induced three individuals of *neogama* to respond to the sound of the organ pipe; but failed with two others. Although the cases in which we succeeded in inducing the moths to respond to sounds to which they do not usually react are few, the fact that we did succeed in a few cases supports our contention that these insects respond only to sounds that have a life significance.

CONCLUSIONS.

1. Our field experiments demonstrate that several different species of *Catocala* moths respond to certain high pitched notes of the Galton whistle; but that they usually do not respond to sounds of low pitch, such as the rumbling of trains, etc.

2. Most specimens responded to those high notes by flying to a nearby tree; but some, and this was especially true of *Catocala relicta*, responded by making quivering movements with its wings.

3. The degree of responsiveness was not the same in all species. Among the least responsive were *C. vidua* and *C. neogama;* at the other extreme were *C. flebilis*, *C. habilis*, and *C. robinsoni*.

4. We do not consider the failure of these moths to respond to certain sounds of low pitch a proof that they do not hear such sounds; indeed, we are inclined to believe that these creatures respond only to such sounds as have a life significance. Three things render this last assumption probable: (1) The fact that *C. unijuga*, which at first did not respond to whistling, did so readily after once a blast of air had been allowed to strike her body simultaneously with the sounding of the whistle; (2) that most of the natural enemies of these moths produce high pitched sounds and trains, and brass bands and other producers of low pitched or coarse sounds do not directly affect the survival of these moths; and (3) by carefully conducted field experiments, we were able to induce three specimens of *C. neogama* to respond to sounds to which the species does not usually react.

REFERENCES.

The following list is not intended to be a complete bibliography of the sense of hearing of insects. It contains only such articles as have been referred to in the body of this paper. An effort has been made, however, to include all papers treating of experiments upon the auditory powers of the Lepidoptera. If any such have been omitted, it is because they have escaped our notice.

Adelung, Nicolai von,
'92 Beitraege zur Kenntnis des Tibialen Gehörapparates der Locusten. Zs. f. wiss. Zool., Bd. LIV., s. 316–349; Pls. XIV–XV.
Andrews, E. A.
'11 Observations on the Termites of Jamaica. Jour. of Animal Behav., Vol. I., pp. 193–228.
Buttel-Reepen,
'00 Sind die Bienen Reflex-maschinen? Experimentelle zur Biologie der Honigbiene. Biol. Centrlbl., Bd. XX., s. 97–108, 130–144, 177–193, 209–224, 289–304.
Deegener, P.
'09 Koennen die Schmetterlinge Hören? Natur, Leipzig, 1909–10, pp. 111–112.
Fielde and Parker.
'04 The Reactions of Ants to Material Vibrations. Proc. Nat. Acad. Sci. Philadelphia, Vol. 56.
Forel, A.
'03 Ants and Some of Their Instincts. Monist, Vol. XIV., pp. 33–66, 177–194.

Graber, V.

'75 Die Tympanal Sinnesorganen der Orthopteren. Denkschriften der K. Akad. der Wissensch, Wien.

'82 Die Chordotonal Sinnesorgane und das Gehör der Insecten. Archiv f. wiss. Anat., Bd. XX., XXI.

Hamann, W.

'09 Haben Schmetterlinge Gehörsinn? Intern. Ent. Zs., Bd. III., pp. 141, 144–146.

Heinrich, Rudolph.

'09 Haben Schmetterlinge Gehörsinn? Entom. Plauderi. Intern. Ent. Zs., Bd. II., s. 275–277.

Henson, V.

'66 Ueber das Gehororgan von Locusta. Zs. f. wiss. Zool., Bd. XVI., h. 2.

Huber, P.

'10 Recherches sur les Moeurs des Fourmis Indigènes. Paris.

Lee, A. B.

'83 Bemerkungen Ueber den Feineren Bau der Chordotonalorgane. Arch. f. Mikr. Anat., Vol. XXIII.

'85 Les Balancers des Dipteres. Rec. Zool. Suisse, Vol. II.

Leydig, Fr.

'55 Zum feineren Bau der Arthropoden. Mueller's Archiv f. Anat. u. Physiologie.

Lubbock, Sir J.

'81 Ants, Bees and Wasps, pp. 221–233.

Montgomery, Th. H.

'10 The Significance of the Courtship and Secondary Sexual Characters of Araneads. Amer. Nat., Vol. XLIV., pp. 151–177.

Omensetter, S.

'12 The Speech of Insects. Proc. Del. County Inst. Sci., Vol. VI., pp. 121–136.

Radl, E.

'05 Ueber das Gehör der Insecten. Biol. Centrlbl., Bd. XXV., s. 1–5.

Richter, Otto.

'09 Koennen Schmetterlinge Hören? Intern. Ent. Zs., Bd. III., s. 124–126.

'10 Gesicht und Gehor bei den Schmetterlingen. Intern. Ent. Zs., Bd. IV., s. 42–43, 45–47, 51–53.

Röber, I.

'10 Gehörsinn bei Schmetterlingen. Zs. wiss. Insectenbiol., Bd. VI., s. 355.

Rothke, Max.

'09 Zum Hörenvermögen der Schmetterlinge. Intern. Ent. Zs., Bd. III., s. 162–164.

Romanes, Geo. J.

'76 Sense of Hearing in Birds and Insects. Nature, Vol. XV., p. 177.

'91 Mental Evolution in Animals, p. 86.

Schaeffer.

'09 Besitzen Insecten Gehörsinn? Intern. Ent. Zs., Bd. III., s. 37–38.

Siebold, von.

'44 Ueber das Stimm und Gehörorgan der Orthopteren. Wiegmann's Archiv f. Naturgeschichte, 10 Jahrg.

Stephan, J.

'12 Tonerzeugende Schmetterlinge. Natur, Munchen, Vol. III., pp. 117–119.

'12 Tonerzeugende Raupen und Puppen. Natur, Munchen, Vol. III., pp. 426–427.

Turner, C. H.,

'07 The Homing of Ants. Jour. of Comp. Neur. and Psy., Vol. V., 367–435.

Weld, LeRoy.

'99 The Sense of Hearing in Ants. Science, n.s., Vol. X., pp. 766–768.

Weinland, E.

'91 Ueber die Schwinger der Dipteren. Zs. f. wiss. Zool., Bd. LI.

Wheeler, W. M.

'10 The Ant, pp. 512–515.

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BIOLOGICAL BULLETIN

SPERMATOGENESIS OF THE HORSE WITH SPECIAL REFERENCE TO THE ACCESSORY CHROMOSOME AND THE CHROMATOID BODY.

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I. INTRODUCTION.

Many interesting things were observed in this study on the spermatogenesis of the horse, but the two points of especial interest and importance are; firstly, the occurrence of a large accessory chromosome, and secondly, the presence of a much smaller though very conspicuous body comparable to the chromatoid body as described by Professor E. B. Wilson ('13) in *Pentatoma.* While the significance of the chromatoid body is problematical, it is a body of extreme interest in this connection on account of its deceptive resemblance to an accessory chromosome. Were it not for the fact that its entire history can be followed out it might lead to serious misinterpretations. Since the occurrence of the chromatoid body in the horse is so constant and its behavior so distinct, and furthermore, since this is the first case among the vertebrates where such a body has been studied in full detail, it is dealt with at some length in this paper.

The significance of the accessory chromosome is of course obvious. It was shown beyond doubt that sex in the pig is determined by such elements (Wodsedalek,' 13). And while embryological material of the horse is not at present available to enable a similar extended study, the presence and unquestionable behavior of the accessory chromosome giving rise to a dimorphic condition among the spermatozoa of this mammal, nevertheless, lend additional support to the chromosome theory of sex determination. The spermatogenesis of the horse resembles to a certain extent the spermatogenesis of the pig (Wodsedalek, '13), and for the purpose of avoiding too much repetition it is treated in a comparative way in the present study.

This investigation was started in the zoölogical laboratory at the University of Idaho, but the main bulk of the work was done at the Wisconsin Biological Station at Madison. And I wish to thank the zoölogy department of the University of Wisconsin for the liberal use of their laboratories, apparatus and material, and the many other courtesies extended me during the summer of 1914.

II. Material and Methods.

The material studied, mainly, was obtained from a horse about a year and a half old. Immediately after the testes were removed from the live animal, small pieces were placed in Bouin's and Gilson's fluids. Sections from various parts of the testes were made from four to ten microns thick, and the material fixed in Bouin's fluid and stained with Heidenhain's iron hematoxylin with acid fuchsin as a counterstain, as in the case of the pig, proved to be the most satisfactory.

Material from an animal about a year old was also studied; but while all the stages including the mature sperm could easily be identified in this material, the chromosomes were very difficult to count on account of being too closely aggregated or lumped together. The finer details of the cells, too, were not as easily made out as in the other material, this being undoubtedly due to the fact that the material was not fixed until about an hour after it was removed from the animal. The chromatoid body, however, was very distinct and could be traced throughout its entire history the same as in the more favorable material. The accessory chromosome, too, could easily be identified, especially through the first spermatocyte division.

III. General Arrangement of the Germ Cells.

The structure of the testes of the horse differs from the pig in that the seminiferous tubules as well as the corresponding cells in the various degrees of development are much smaller and the interstitial cells are much fewer in number. The continuous network of connective tissue walls is present, but the chambers formed by this network and filled with coiled tubules are much larger in the horse and, therefore, a section through one of the chambers as a rule reveals many more sections of the tubules. These chambers in the horse testes do not show the same regularity in size as is the case in the pig testes. In some cases a group of over a hundred sections through the tubules are surrounded by the connective tissue wall and then again a count of only a dozen or so can be made. The arrangement of the cells in the tubules is similar to that of the other well-known mammals, particularly the pig.

IV. Spermatogonia.

As a rule the spermatogonia lie in a single layer next to the wall of the tubule, though occasionally some of the cells are crowded out. At times the cells are far apart, in which case they are flattened out on the tubule wall. The cells also differ considerably in size and appearance, depending on the stage of development they are in (Figs. 1–3).

During the resting stages a large nucleolus is invariably present. As a rule it assumes a somewhat heart-shaped appearance; especially is this true in the larger cells and in those in which the chromosomes are beginning to form. A much smaller spherical nucleolus also appears to be fairly constant (Figs. 1

and 2). Other nucleoli varying considerably in size, shape, and number also appear in some of the cells (Fig. 2).

Before the chromosomes begin to form the cells increase greatly in size (Fig. 2). At the conclusion of the resting stage numerous large chromatin granules appear which arrange themselves along fine threads in an entangled mass. The chromosomes soon become distinct and while, as a rule, a count is impossible on account of the overlapping and massing together of the chromosomes, [the mitotic stages were abundant and many distinct counts could be made. Thirty-seven chromosomes appear in the late prophases of the spermatogonial division (Figs. 4 and 5). Thirty-six of these are variously shaped, mainly oblong, and differ somewhat in size. One which is much larger is, as a rule, somewhat triangular or heart-shaped. This is the accessory chromosome and is the same thing as the large nucleolus which appears in the resting stages. That is certain, as the body can easily be traced through the various stages of growth. This condition is similar to that found by Guyer ('10) in man, and Wodsedalek ('13) in the pig. Ordinarily about two thirds of the chromosomes arrange themselves in a ring which encircles the remaining one third. The accessory chromosome may be found anywhere within the mass, and occasionally occurs outside of the main ring, but never far removed from the other chromosomes. During division each chromosome divides in two. The accessory as a rule divides a little in advance of the other chromosomes (Figs. 6 and 7).

The spermatogonia in this, as well as other stages, vary somewhat in size (Figs. 6 and 7). In the smaller cells the cytoplasm appears denser and the chromosomes are more crowded together.

V. PRIMARY SPERMATOCYTES.

1. *Resting Stage.*

The primary spermatocytes arising from the final spermatogonial division in the early resting stage are usually smaller than the spermatogonia immediately preceding and during the division stages. After the disintegration of the chromosomes the nucleus appears much clearer than it does in the later growing stages. The large nucleolus is again very conspicuous and easy to distinguish from other nuclear bodies when such are present (Figs. 8–11). The small spherical nucleolus again appears to be fairly constant, though at times it is difficult to distinguish it from the other bodies.

2. *Synizesis and Growth Period.*

After a brief period of rest the cells begin to increase in size. For some time the nucleus appears much the same as it does in the resting stage of the spermatogonia (Fig. 8). Later it becomes more granular and the linin fibers become more distinct (Fig. 9). Soon after, the chromatin threads become massed in the center of the nucleus (Fig. 10), and later the nuclear wall expands and the entire mass passes to one side of the nucleus, leaving a large clear area in the remaining portion (Fig. 11). This condition is much the same as in the pig except that in that animal the nucleoli were invariably found within the mass of threads and in a position nearest to the nuclear wall, while in the horse the nucleoli are almost invariably within, or next to the clear area (Fig. 11). The nuclear wall in this stage is often very irregular, especially next to the clear portion of the nucleus.

Shortly after the collapse of the chromatin material, the threads pair and appear in about half the original number and twice as thick (Figs. 10–12). There is considerable evidence that pairing of the threads takes place by parasynapsis, and nothing was observed which would indicate that it takes place otherwise; but this phase of the problem demands more study and no positive statement can be made in regard to it at this time. The entire mass of threads then moves toward the center and the large clear area disappears (Fig. 12). The large nucleolus passes toward the periphery of the nuclear wall and the threads soon become evenly distributed. Then follows the period of growth during which time both the nucleus and cytoplasm increase greatly in size (Figs. 13 and 14). The chromatin threads and the large nucleolus also increase considerably in size. It is between the synaptic stage and the fully developed spireme stage that the chromatoid body makes its appearance (Figs. 12–14).

3. *Reduction Division.*

Nineteen chromosomes appear in the late prophase or early metaphase stages of the primary spermatocyte (Figs. 15–18). Eighteen of these are the ordinary chromosomes or autosomes and the other is the accessory chromosome. The accessory in this case is practically always found outside of the main mass of chromosomes, either in close contact with them (Figs. 16 and 18), or a short distance away (Figs. 15 and 17). The large size of the eighteen autosomes which are about four times the size of the chromosomes in the spermatogonia indicates that they were formed by the growth and pairing of the thirty-six autosomes found in those cells, while the accessory remains unpaired, making a total of nineteen.

In these cells as in the case of the spermatogonia the chromosomes are frequently bunched together, making an accurate count difficult and often impossible. However, mitotic stages particularly of the first and second spermatocyte divisions were very numerous and among the thousands of cells in mitosis examined several hundred definite counts were made. Figs. 19–29 show the accessory in characteristic positions in the metaphases of division of the primary spermatocyte. The heart-shaped body always passes toward one pole in advance of the other chromosomes and frequently may be found at the pole before the other chromosomes have divided (Figs. 28 and 29). The chromatoid body which is spherical in shape and much smaller than the accessory is also invariably present and very conspicuous. As a rule it is in the spindle, and in a large majority of the cases goes in the direction opposite from the accessory (Figs. 21, 22, 23, 24, 25, 27 and 28), though this behavior is by no means constant, for occasionally it is found with the accessory on the same side of the equatorial plate (Figs. 20, 26 and 29).

When the large, apparently quadrivalent chromosomes divide, the resulting chromosomes are somewhat larger than the chromosomes of the spermatogonia. Immediately after the chromosomes divide they unite in twos (Fig. 29) so that at the time of their arrival at the poles they do not number eighteen, but only nine or exactly one half that number (Figs. 30–35). Additional proof that such a second pairing of the chromosomes occurs lies in the fact that the resulting nine chromosomes are not one half the size of the original eighteen chromosomes of these cells, but exactly of the same size and apparently quadrivalent. This quadrivalent nature becomes obviated after the division of the secondary spermatocyte, where the resulting chromosomes are bivalent. The primary spermtocyte division is undoubtedly the reduction division and, speaking in terms of univalence, one of the resulting secondary spermatocytes receives eighteen chromosomes and the other eighteen plus the accessory. In terms of bivalence the one type of secondary spermatocytes receive nine chromosomes and the other nine plus the accessory (Figs. 30–33).

VI. Secondary Spermatocyte.

No resting stage occurs in the secondary spermatocyte, a condition similar to that frequently found in the spermatogenesis of the pig. The second pairing of the chromosomes also takes place here as it does in the pig (Wodsedalek, '13), man (Guyer, '10), and opossum (Jordan, '11). In the pig, however, this pairing takes place much later, never before the cell is completely divided. The secondary spermatocytes divide soon after they are formed and not infrequently the spindles are formed in the two cells resulting from the first spermatocyte division while they are still in close contact. Nine chromosomes arrange themselves in the equatorial plate for division in the one type of secondary spermatocyte (Figs. 42–45), and nine plus the accessory in the other (Figs. 34–38). All of the chromosomes, including the accessory when it is present, divide in these cells (Figs. 36–47). The accessory usually lies a little to one side of the other chromosomes (Figs. 34 and 35), and again, as in the spermatogonia, divides a little in advance of the other chromosomes (Figs. 36–38). This may be due to the partial separation of the two halves of this body even long before the other chromosomes line up for division in this stage (Figs. 21–33). The heart-shape it assumes during the later stages of the primary spermatocyte division and retains during the secondary spermatocyte, is no doubt due to a partial separation at one end of the two components. The chromatoid body remains very conspicuous (Figs. 35–55).

VII. Spermatids.

The division of the secondary spermatocytes gives rise in the one case to spermatids containing nine chromosomes (Figs. 46, 55 and 56), and in the other case nine plus the one accessory or ten chromosomes (Figs. 39–41). All of the chromosomes except the accessory are bivalent in nature (Figs. 23–41, 54–56), so that in reality we have the equivalent of eighteen chromosomes in the one kind of spermatid and eighteen plus the accessory in the other. All of the foregoing evidences indicate that eighteen is the reduced number of chromosomes.

The accessory is usually out of the main mass of chromosomes (Figs. 40 and 41). Soon after the secondary spermatocyte divides the chromosomes become massed together and the nuclear wall begins to form (Figs. 57–59). In the resting stage half the spermatids contain a large nucleolus which is the same thing as the accessory chromosome, since it can be traced through all the stages in the formation of the nucleus (Figs. 63–65). The other half of these cells lack such a body (Fig. 62). In some cases this nucleolus persists in the developing stages of the spermatozoön (Figs. 72 and 73). Especially is this true in material which has not been destained too much. In favorably stained material the centrosome surrounded by a clear layer can be seen within the centrosphere (Figs. 64–66). The chromatoid body is still very distinct (Figs. 55–67).

VIII. Development of the Spermatozoa.

The development of the spermatozoön in the horse is essentially the same as the development of the spermatozoön in the pig (Wodsedalek, '13). The centrosome surrounded by a clear area emerges from the sphere (Fig. 67) and soon divides into two spherical bodies (Fig. 68). The anterior one comes in contact with the nuclear wall, while the posterior one which remains spherical passes down the developing axial filament (Figs. 69, 70, 71, 73, 74). This posterior body which is quite small never assumes the shape of a ring as it does in the pig. It passes far down the filament and often no trace of it is left (Fig. 79). Then again it retains a size just enabling detection (Fig. 73). As a rule, however, a sufficient amount of it is left to be sloughed off as in the case of the pig (Figs. 74, 75, 77 and 84). The chromatoid body is, in rare cases however, also seen on the filament and in such cases apparently fused with the posterior centrosome (Figs. 71 and 76). It is invariably sloughed off before the spermatozoön is fully developed (Figs. 77 and 86).

Shortly after the centrosome divides the nucleus begins to elongate and at the same time migrates toward one end of the cell, so that soon practically all of the cytoplasm is found at the posterior end of the developing sperm (Figs. 67–75). As the acrosome-end of the nucleus comes in contact with the cell-wall no break in the latter is ever noticeable, and the apparent backward pull exerted by the mass of cytoplasm causes the cell-wall to become closely applied to the nuclear wall where it undoubtedly persists as an additional covering of the sperm-head (Figs. 73–79). This supposition that the cell-wall forms an additional covering of the sperm-head is based on two observations; firstly, there is no evidence that the head penetrates the cell-wall, and secondly, the covering of the sperm-head is much thicker after the entire mass of cytoplasm lies at its posterior end (Figs. 73–79). This fact gives one the impression that the distinctly noticeable change in the thickness of the head covering is brought about by the fusion of the two walls. It is also obvious that the cell-wall is not entirely consumed in forming the external covering of the head of the sperm, for it can always be seen surrounding the anterior portion of the axial filament and extending far down into the mass of cytoplasm which is apparently squeezed out of it and about to be thrown off (Figs. 77–79). In the final stages it becomes closely applied to the axial filament and one may safely conclude that the axial envelope is at least partly formed by the portion of the cell-wall extending down from the head (Figs. 77, 79 and 85). This same condition was found to exist in the pig.

When the developing sperms reach the stage represented in Fig. 73 they become attached in clusters to the large nurse cells. As the sperms develop the cytoplasmic mass of the nurse cells decreases. Just as the mass of cytoplasm is being thrown off by the developing spermatozoa, the latter leave the nurse cells and become embedded in the layer of cytoplasm composed of the cast-off masses, apparently nursing on the material so that little of it, if any, goes to waste.

Every stage in the sloughing off of the cytoplasmic mass can easily be observed (Figs. 77–86). When these masses of naked cytoplasm are completely sloughed off they assume a rounded shape and if the chromatoid body is present they might, at first sight, be mistaken for minute cells with the chromatoid body as the nucleus. And I feel that Wilson ('13), in speaking of this condition in *Pentatoma*, is absolutely correct when he says, "I also think it probable that the bodies that have been described as 'degenerating cells' in the late spermatid-cysts by some observers are identical with the protoplasmic balls here described."

Among the cast-off balls four different types can be observed (Figs. 80–83). One type contains a small body which apparently is the remnant of the posterior centrosome (Fig. 80); another type is clear and one is led to believe that in such a case the centrosome was entirely consumed (Fig. 81); another shows the same condition regarding the small body but contains the chromatoid body (Fig. 86); and still another contains both the chromatoid body and the much smaller centrosome remnant (Fig. 82). Later when the spermatozoa are fully developed the roundish masses become irregular in shape and finally begin to disintegrate. The disintegration is characterized by the breaking-up of the masses into small particles and by the appearance of many deeply staining bodies and globules which vary considerably in size (Fig. 83).

Occasionally in the last stages of the disintegration of the cytoplasmic material and also when the material entirely disappears there may be seen small, deeply staining bodies identical in size and appearance to the chromatoid body and one is led to believe that it is the same thing (Figs. 83 and 84). If it is the same thing the fact throws some light on its durable consistency.

The mature spermatozoön in general resembles that of the pig, except that it is smaller, and the head is thinner at the anterior end and thicker at the posterior end. The entire nucleus enters into the formation of the head and the contents become homogeneous and intensely staining.

IX. Variation in Size of Adult Spermatozoa.

The spermatozoa of the horse like those of the pig vary considerably in size and many careful measurements show that they, too, are of two distinct types, the one being much larger than the other. Mature specimens which were free in the lumen of the tubule and parallel to the objective, were selected at random and outline sketches of six hundred heads enlarged (× 2,000) were made with the aid of a camera lucida. The lengths of the sketches were then measured and recorded in quarter millimeters. It can be seen from Fig. 1 in the text that two separate types of spermatozoa exist; the greatest number of the one kind measuring 9.5 mm., and of the other 11 mm. I think it is safe to assume that this dimorphic condition in the size of the mature spermatozoa is due to the accessory chromosome. The increased size in the one type is due presumably to the presence of that element.

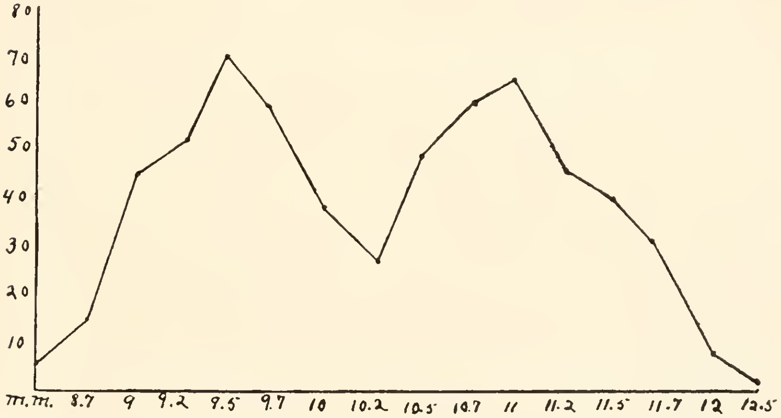


Fig. 1. Diagram showing the variation in size among six hundred mature horse spermatozoa. Figures at the left give the numbers of individuals belonging to each type. Figures at the bottom give the lengths of the heads of the spermatozoa in millimeters, magnified two thousand times.

A similar dimorphic condition was found to exist among the spermatozoa of the pig; one type measuring from 11 to 12 mm., and the other from 14 to 14.5 mm. (Wodsedalek, '13). Size dimorphism also exists in the adult spermatozoa of *Anasa tristis* (Faust, '13). This of course is exactly what would be expected since the spermatogenesis studies of this form indicate that one half of the spermatozoa receive one more chromosome each than the other half (Paulmier, '99).

X. Migration of the Developing Spermatozoa.

In the very beginning of the transformation of the spermatids into spermatozoa when the acrosome takes a position on the nucleus opposite the dividing centrosome (Fig. 68), the anterior end of the sperm-cell which bears the acrosome, invariably points in the direction of the tubule wall and the Sertoli or nurse cells. Long before the tail is sufficiently developed to aid in locomotion these sperm-cells move a short distance and come in contact in bunches with the nurse cells. There apparently exists some attraction between the nurse cells and the nuclei of the sperm cells in that stage of development. In this first stage of migration only the nucleus appears to be attracted while the cytoplasm exhibits a tendency to remain in place. The fact that the cytoplasm does have a tendency to remain in place while the nucleus or sperm-head moves forward undoubtedly accounts for the posterior position that the entire mass of cytoplasm assumes with respect to the sperm-head (Figs. 69–79). This change in position of the cytoplasm to the posterior end of the developing cell occurs simultaneously with the migration of the cell, which is further evidence for such an assumption. The nucleus is apparently attracted with sufficient intensity to enable it to pull the entire cytoplasmic contents after it to a certain extent. In the later stages the movement of the sperm-head deeper into the cytoplasm of the nurse-cell is probably facilitated by the flagellum-like motion of the filament which extends a considerable distance out of the cell (Figs. 75–79). Later, when the spermatozoa are almost fully developed and slough off the balls of naked cytoplasm (Figs. 85 and 86) they back out away from the nurse-cells, becoming embedded in the cast-off material where they remain scattered until they are fully developed and then become free in the lumen of the tubule. This indicates that the sperms in that stage of development are attracted more by their own thrown-off material than by the rather scanty contents of the nurse cells, which are then very much collapsed, owing to the large number of developing sperms which they have nourished. This migration of the sperm backward is probably nothing more than a chemotactic response to the food contained in the balls of cytoplasm.

XI. The Chromatoid Body.

The behavior of the chromatoid body in the horse bears a striking resemblance to the behavior of the chromatoid body described in *Pentatoma* (Wilson, '13). Dr. Wilson treats the subject at considerable length in his paper and therefore much of the detail concerning this body in the horse may safely be omitted. However, all the more essential features will be presented here since this is the first case among the vertebrates, according to my knowledge, where such an element has been studied in full detail. The reader is advised to familiarize himself with Professor Wilson's article in order to appreciate fully the surprising similarity existing in the behavior of the chromatoid body in such diverse classes of animals as the insects and the mammals.

In speaking of the chromatoid body Professor Wilson ('13) says in part, "As seen during the growth-period and the spermatocyte-divisions it is of rounded form, dense and homogeneous consistency, and after double staining with hæmatoxylin or safranin and light green is at every stage colored intensely blue-black or brilliant red, precisely like the chromosomes of the division-period or the chromosome-nucleoli of the growth-period. In the first spermatocyte-division it may lie anywhere in the cell, sometimes almost at the periphery, but is often close beside the chromosomes. In the latter case it usually lies in, on or near the spindle, lags behind the chromosomes during the anaphases, and in later stages is found near one pole, presenting an appearance remarkably like that of an accessory chromosome (Figs. 8–10). For such in fact I mistook it, even after the discovery that a similar body is often also seen near one pole in the *second* division (Figs. 22, 23); for I supposed this might be a case like that of *Ascaris megalocephala*, where, according to Edwards ('10) the X-chromosome may pass undivided to one pole in either the first or second division. The resemblance is indeed most deceptive; and these division-figures have often been exhibited to other observers as "a remarkably clear demonstration of an accessory chromosome" without at first arousing the least suspicion of the hoax.

"The body in question is nevertheless neither an accessory nor any other kind of chromosome; though this did not become wholly certain until after a study of the entire spermatogenesis. It is in fact of protoplasmic origin, first appearing early in the growth-period outside the nucleus, whence it may be followed uninterruptedly through all the succeeding stages until it is finally cast out of the spermatozoön. Upon dissolution of the nuclear membrane it is left lying near the chromosomes, passes without division into one of the daughter-cells in each of the spermatocyte-divisions, and thus enters but one fourth of the spermatids."

In the horse the chromatoid body is of a spherical shape and also of a dense and homogeneous consistency, and stains exactly like the chromosomes of the division stages or the chromosome-nucleoli of the growth-period. It is invariably surrounded by a clear area. It makes its appearance in the stages immediately following synizesis and apparently attains its full size rather abruptly, for as a rule even in the earlier growing stages, if it is present at all, it possesses its full size, although in some cases it was found to be somewhat smaller (Fig. 13). Occasionally, in the earliest stages immediately following synizesis one or two very small bodies within clear vacuoles could be detected (Fig. 12). Two such small bodies are extremely rare and even the single minute bodies showing the very beginning of the chromatoid body are not numerous; however, it is quite certain that the body practically always originates as a single element. When the cells attain their maximum size the chromatoid body is invariably present and possesses its full size which makes it very conspicuous (Fig. 14).

The chromatoid body may be seen anywhere within the cytoplasm, either near the nucleus or far from it. Sometimes it appears to be in fairly close contact with the cell-wall (Fig. 14). When the nuclear wall disappears and the chromosomes come into full view, it may again be found anywhere in the cytoplasm. Later when the chromosomes arrange themselves for division in the equatorial plate it most generally takes a position near them (Figs. 15 and 18), and when the spindle is formed, in a large majority of the cases, it takes a position in, on or near the spindle (Figs. 21–28) as is the case in Pentatoma (Wilson, '13). This, however, is not always the case, for occasionally it is far away from the spindle (Figs. 20 and 29).

It was in the primary spermatocyte division that the chromatoid body was first observed. It attracted my attention at the very first glance at the material under low power of the microscope and its constant appearance in this stage led me to suppose, at first, that it may be an accessory chromosome. Soon, however, the large, heart-shaped accessory was discovered and for some time I had the impression that this was the *X*-chromosome and the small spherical body the *Y*-chromosome. This temporary, erroneous impression was obtained through the peculiar fact that in about ninety per cent. of the cases the chromatoid body passes over to the half of the dividing cell opposite from that containing the large accessory, and in almost a hundred of the first mitotic stages examined not a single case was noticed in which the spherical body was on the same side of the equatorial plate with the accessory chromosome. Even when the first case in which both of the bodies were seen on their way to the same pole was observed, the matter was not taken very seriously. Later, however, when more such cases were seen, my suspicion was aroused and further observations convinced me that besides the supposed *y*-chromosome a body identical to it was present. And it was not until the entire history of the body could be traced from the growth-period to the casting-off of the mass of cytoplasm in the final stages of the developing spermatozoön, that I was absolutely certain that the suspicious looking element and the supposed *y*-chromosome were one and the same thing, namely, the chromatoid body, first described by Wilson in insects.

In exceptionally rare cases, one (Figs. 23 and 26) or two other small, deeply staining bodies within clear vacuoles occur in the cytoplasm (Fig. 27). However, in cases where such bodies do occur, there is no appreciable difference in the size of the chromatoid body and therefore it is difficult to determine whether such bodies are simply portions split off from the chromatoid body, or whether they originate separately. In only three cases did I observe two bodies apparently of equal size and smaller than the profoundly constant chromatoid body (Fig. 54). Were such cases more numerous one might assume that such bodies are the components of the chromatoid body, but since such bodies are of such extremely rare occurrence no definite statement can be made in regard to them.

When the primary spermatocyte divides the chromatoid body is practically always found in only one of the resulting cells (Figs. 30–33) and in a large majority of the cases it is found in the cells which do not contain the accessory chromosome (Figs. 30, 31 and 33). This, however, is not universal, for in some cases at least, it is found in the same cell which contains the accessory (Figs. 32 and 35); and it has also been seen in the division stages of such a type of secondary spermatocyte (Figs. 38 and 39) as well as in the spermatid resulting from such a division (Fig. 41). In the anaphase of the secondary spermatocyte division the body is usually seen lagging on the spindle threads behind the masses of chromosomes (Figs. 39, 48, 49, 51 and 52); occasionally, however, it is seen at the pole (Fig. 51). After the division is complete the body usually lies far out in the cytoplasm (Figs. 41 and 57), and in rare cases only, is it seen in close contact with the nucleus. Figure 58 represents an extreme case of that nature, and it appears that such a condition is brought about when the chromatoid body bears a relation to the chromosome as is represented in Fig. 51. Sometimes two bodies (Fig. 60), though not always of the same size, appear in the spermatid.

In the late resting stages of the spermatid the body may again be found anywhere in the cytoplasm (Figs. 62–67), at times near the nucleus (Fig. 62). Sometimes it is found in close contact with the centrosome (Figs. 63 and 66) and in only rare cases it is found on the axial filament, giving the impression that it is fused or in close contact with the posterior centrosome (Figs. 72 and 76). Later, however, it leaves the filament and lies freely in the cytoplasm (Figs. 74, 75, 77, 86). In the final stages of the developing spermatozoön when the cytoplasmic mass is cast off, the chromatoid body when present is invariably thrown off with it (Figs. 82 and 86). It is certain that the chromatoid body does not contribute in any visible way to the formation of the spermatozoön. The foregoing facts also indicate that great care must be exercised in interpreting the significance of bodies which appear like chromosomes, but really are something entirely different and no positive statements can be made regarding their meaning unless their entire history can be definitely traced.

It is very probable that a body similar to the chromatoid body in the horse also exists in the pig. In speaking of a small chromatin body which frequently occurs in the first spermatocyte division of the pig (Wodsedalek, '13), I make the following statement: "Occasionally a small chromatin body is present in this first spermatocyte division (Figs. 28, 31, 32, 35 and 37). Fig. 31 shows such a body passing to the same pole with the accessories, in advance of the other chromosomes. Fig. 32 represents an earlier stage of much the same thing. In Fig. 35 it can be seen passing to the opposite pole, and Fig. 37 represents an extremely rare case where two such bodies are present, one somewhat larger, passing to either pole, even in advance of the two accessory chromosomes. While the small body can be seen frequently, as a rule no such element can be detected, and while it may possibly be comparable to the small pair of chromosomes found so constantly in some of the Tracheata, my present data on its irregular occurrence and behavior do not permit a conclusion regarding its significance."

Further investigation regarding the body in question in the pig will be taken up presently. It might also be mentioned here that the chromatoid body is present in the germ-cell of the bull. A complete account of its behavior in that animal will be published later.

XII. Summary.

1. Thirty-seven chromosomes differing somewhat in size occur in the spermatogonia. One, the accessory, is distinctly larger than the others.

2. In the spermatogonial division the accessory divides a little in advance of the other chromosomes.

3. Nineteen chromosomes appear in the primary spermatocyte division, of which eighteen are evidently bivalent and the other is the accessory.

4. In the secondary spermatocyte division the heart-shaped accessory passes undivided to one pole in advance of the other chromosomes.

5. The primary spermatocyte division is evidently the reduction division, giving rise to two different types of secondary spermatocytes; one with the accessory and the other lacking it.

6. There is no resting stage following the first spermatocyte division.

7. A second pairing of the chromosomes takes place so that only one-fourth the original number of chromosomes appear for division in the secondary spermatocyte.

8. The accessory chromosome divides in the secondary spermatocyte division a little in advance of the other chromosomes the same as it does in the spermatogonia.

9. The one type of secondary spermatocyte, which contains the accessory, gives rise to two spermatids, each containing the accessory and nine bivalent chromosomes.

10. The other type of secondary spermatocyte, which lacks the accessory, gives rise to two spermatids, each containing only the nine bivalent chromosomes.

11. In terms of univalence, then, one type of spermatid receives eighteen chromosomes plus the accessory and the other type receives only the eighteen ordinary chromosomes.

12. In view of the foregoing facts, two different types of spermatozoa, equal in numbers, are produced in the horse; the one type contains in addition to the ordinary chromosomes the accessory, and is apparently the female determining spermatozoön.

13. Actual measurements of six hundred mature spermatozoa reveal the interesting fact that two distinct types of spermatozoa as regards size are produced, the one being much larger and presumably the one which bears the accessory chromosome.

14. The dimorphic condition among the spermatozoa of the horse lends additional support to the chromosome theory of sex determination.

15. The developing spermatozoa invariably cast off a mass of cytoplasm.

16. A chromatoid body, which simulates the appearance of a

y-element in the primary spermatocyte division stages, makes its appearance during the growth period and can be traced forward until it is finally thrown off with the ball of cytoplasm in the developing spermatozoön. It does not contribute in any visible way to the formation of the spermatozoön.

LITERATURE CITED.

Faust, E. C.

'13 Size Dimorphism in Adult Spermatozoa of Anasa tristis. Biol. Bull., Vol. XXV., No. 5, Oct., 1913.

Guyer, M. F.

'10 Accessory Chromosomes in Man. Biol. Bull., Vol. XIX., No. 4.

Jordan, H. E.

'11 The Spermatogenesis of the Opossum (Didelphys virginiana) with Special Reference to the Accessory Chromosome and the Chondriosomes. Archiv für Zellforschung, 7. Band, 1. Heft.

Paulmier, F. C.

'99 The Spermatogenesis of Anasa tristis. Jour. Morph., XV., supl., pp. 223–272.

Wilson, E. B.

'13 A Chromatoid Body Simulating an Accessory Chromosome in Pentatoma. Biol. Bull., Vol. XXIV., No. 6, May, 1913.

Wodsedalek, J. E.

'13 Spermatogenesis of the Pig, with Special Reference to the Accessory Chromosomes. Biol. Bull., Vol. XXV., No. 1, June, 1913.

'13 Accessory Chromosomes in the Pig. Science, n.s., Vol. XXXVIII., No. 966, pp. 30–31.

EXPLANATION OF PLATES.

Plate I.

(All of the drawings were made with the aid of a camera lucida, × 2,400.)

Fig. 1. Early spermatogonial cell showing a large triangular nucleolus and two small nucleoli, one of which is spherical. Other cells in the same stage often show many more nucleoli.

Fig. 2. Resting stage of a full grown spermatogonial cell showing the large triangular nucleolus and several small nucleoli, one of which is spherical and can frequently be detected.

Fig. 3. Prophase of a spermatogonial division in which the chromosomes are still rather indistinct.

Figs. 4 and 5. Late prophase of spermatogonial division showing thirty-six ordinary chromosomes and the large accessory which can easily be distinguished.

Figs. 6 and 7. Metaphase of division in a spermatogonium showing the accessory dividing in advance of the other chromosomes. In Fig. 6 the cell appears smaller and the chromosomes are more crowded.

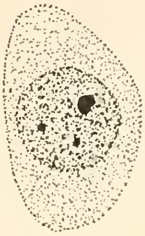
Figs. 8 and 9. Early and late resting stages of a primary spermatocyte, respectively. Both show the large and the small nucleolus.

Fig. 10. Primary stage just before synizesis showing a mass of fine threads and the two nucleoli.

Fig. 11. Primary spermatocyte in synizesis showing the nucleoli in a characteristic position out of the mass of threads.

Fig. 12. Primary spermatocyte following synizesis and synapsis. The threads scatter about in the nucleus.

Figs. 13 and 14. Spireme stage of a primary spermatocyte showing increase in size of the cytoplasm, nucleus and the large nucleolus, and the beginning of the chromatoid body.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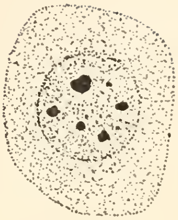
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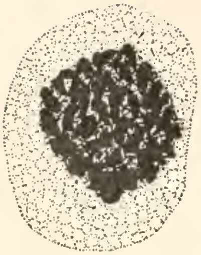
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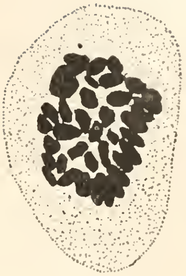
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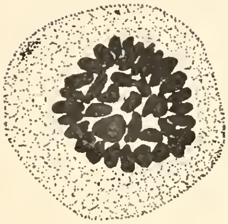
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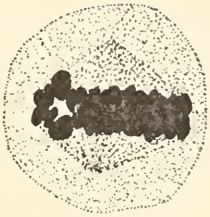
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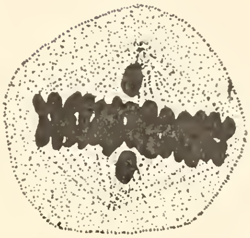
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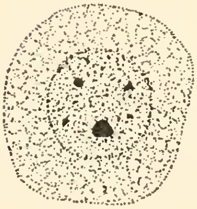
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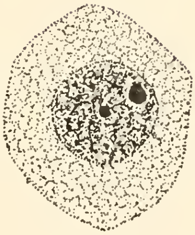
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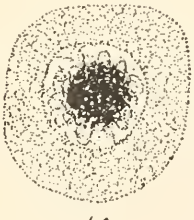
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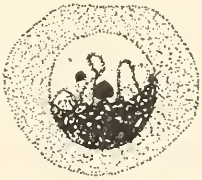
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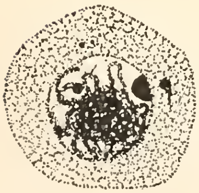
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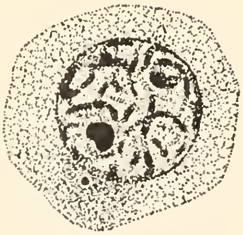
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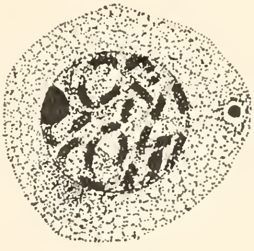
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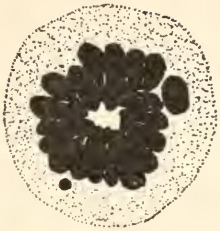
PLATE II.

FIGS. 15–18. Late prophases of primary spermatocytes showing eighteen large chromosomes, the accessory a little off to one side and the conspicuous chromatoid body anywhere in the cytoplasm. Fig. 16 shows a characteristic bunch of chromosomes in which a count is impossible.

FIGS. 19–26. Metaphase of division in primary spermatocyte, showing the accessory chromosome in characteristic positions passing to the pole, and also the chromatoid body. Figs. 20 and 26 show the chromatoid body with the accessory on the same side of the equatorial plate. Figs. 23 and 26 show also an extra small body.



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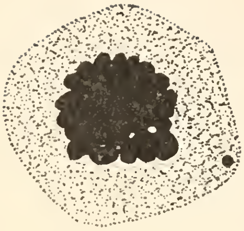

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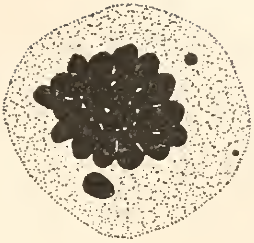

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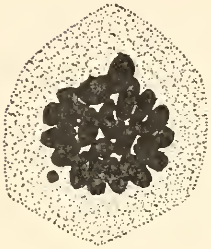

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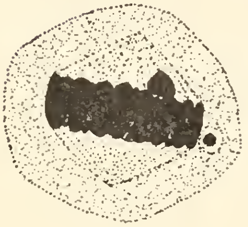

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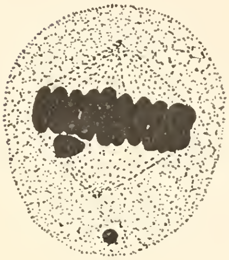

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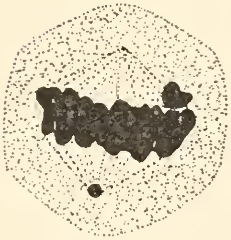

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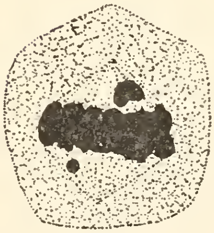

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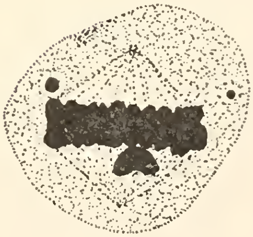

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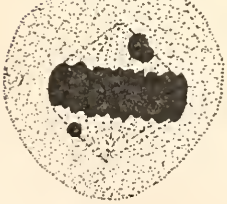

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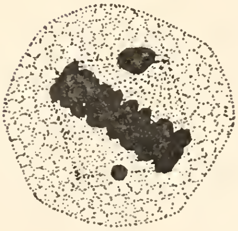

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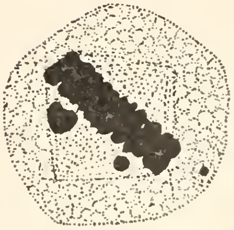

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PLATE III.

FIGS. 27, 28, AND 29. Metaphase of division in primary spermatocyte showing the accessory chromosome and the chromatoid body. Fig. 27 shows two other small and deeply stained bodies. Fig. 28 shows the accessory at one pole and the chromatoid body at the other. Fig. 29 shows the accessory at the pole, and the chromatoid body off the spindle and near the periphery of the cell.

FIGS. 30 and 31. Late anaphase of division in primary spermatocyte showing nine large chromosomes and the accessory at one pole, and nine large chromosomes and the chromatoid body at the other.

FIG. 32. Late anaphase of division in primary spermatocyte, showing nine chromosomes at one pole, and nine chromosomes, the accessory, and the chromatoid body at the other.

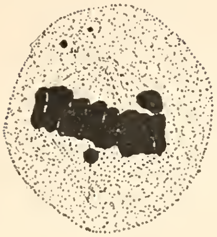
FIG. 33. Two resulting cells of a primary spermatocyte division, one containing the accessory chromosome and the other the chromatoid body.

FIGS. 34 and 35. Late prophase of division in a secondary spermatocyte which received the accessory chromosome. Cell represented in Fig. 35 also shows the chromatoid body.

FIGS. 36, 37, AND 38. Metaphase of division in the secondary spermatocyte showing the division of the accessory in advance of the other chromosomes. Fig. 38 also shows the chromatoid body near the periphery.

FIG. 39. Late anaphase of division in a secondary spermatocyte which received the accessory chromosome, nine apparently bivalent chromosomes and the large accessory can be seen at either pole and the chromatoid body is between the two masses of chromosomes.

FIGS. 40 AND 41. Spermatid showing nine bivalent chromosomes and the accessories. Fig. 41 also shows the chromatoid body.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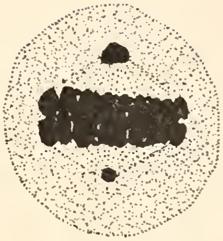
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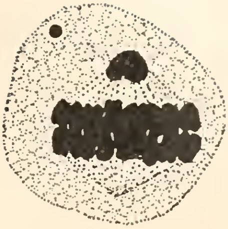
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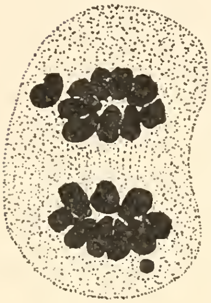
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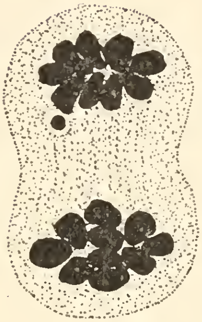
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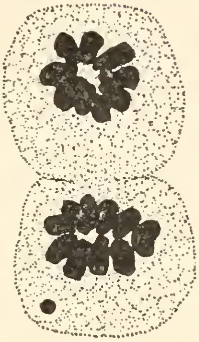
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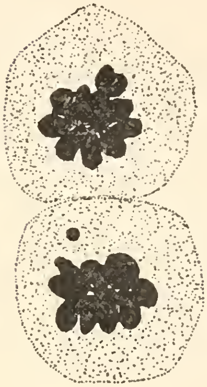
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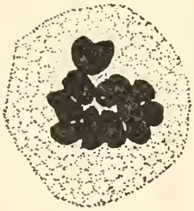
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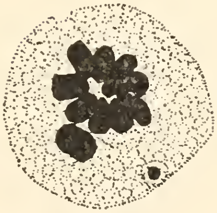
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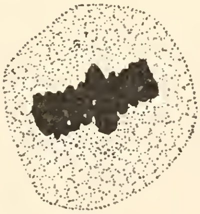
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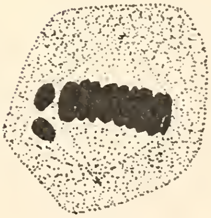
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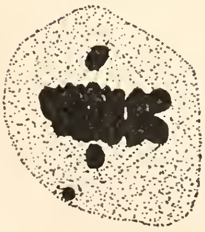
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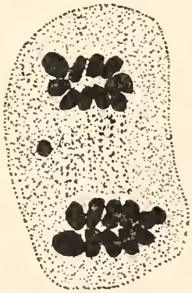
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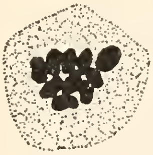
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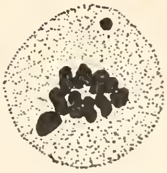
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PLATE IV.

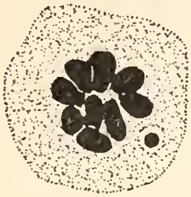
FIGS. 42, 43, AND 44. Late prophase of division in a secondary spermatocyte which did not receive the accessory chromosome, showing only the nine ordinary chromosomes. Figs. 42 and 43 show also the chromatoid body.

FIGS. 45 AND 46. Early metaphase of division in a secondary spermatocyte which did not receive the accessory chromosome. Fig. 45 shows the chromatoid body off the spindle.

FIGS. 47–54. Late anaphase of division in secondary spermatocytes showing various positions of the chromatoid body when it is present. Fig. 47 shows also a small body in addition to the chromatoid body. The cell is one which did not receive the accessory chromosome. In the cell represented in Fig. 50 the chromatoid body was absent and in Fig. 54 two bodies may be seen.

FIGS. 55 AND 56. Spermatid showing nine bivalent chromosomes which is one of the resulting cells of the division of a secondary spermatocyte which did not receive the accessory chromosome. Fig. 55 shows the chromatoid body.

FIG. 57. Characteristic massing of the chromosomes just before the nuclear wall of the spermatid is formed.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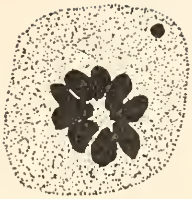
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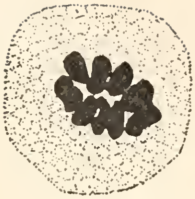
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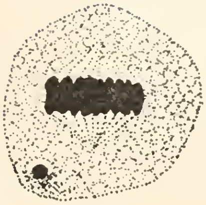
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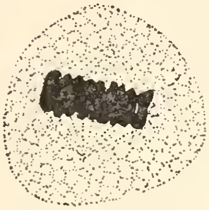
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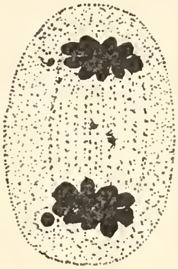
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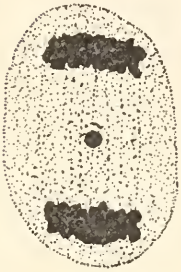
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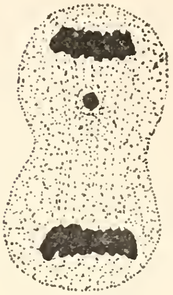
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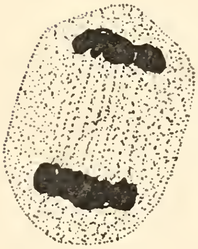
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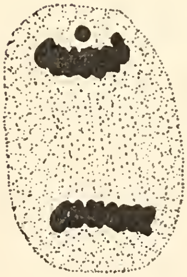
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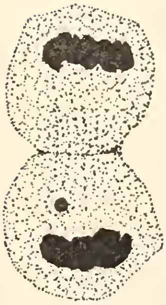
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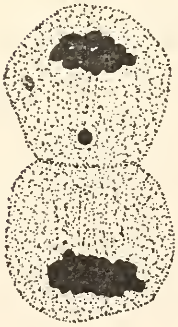
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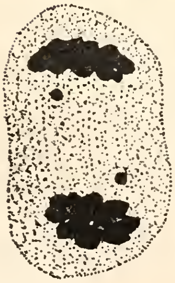
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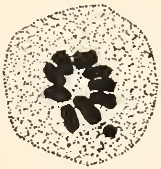
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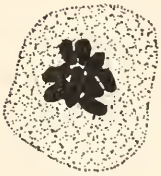
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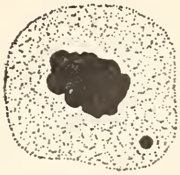
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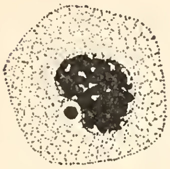
Plate V.

Figs. 58–61. Early spermatids showing the characteristic structure of the nucleus, and the position of the chromatoid body. Fig. 60 shows two bodies of practically the same size as the chromatoid body.

Figs. 62–67. Resting stage of the spermatid. Figs. 62–65 show the large nucleolus or the accessory chromosome. Fig. 62 shows the chromatoid body near the nucleus; Fig. 63 shows it very near the centrosome which is out of its sphere; Fig. 65 shows it at the periphery of the cell; Fig. 66 shows it near the centrosphere; and Fig. 67 shows it far from the centrosphere out of which the centrosome had just emerged.

Figs. 68–73. Early stages of the developing spermatozoön. Fig. 68 shows the divided centrosome, the very beginning of the axial filament, and the acrosome which had migrated to the anterior end of the nucleus or sperm-head; Fig. 69 shows the posterior centrosome passing down the axial filament, and the chromatoid body far down in the cytoplasm away from the filament; Fig. 70 shows the same thing except that the chromatoid body is not present; Fig. 71 shows the chromatoid body near the posterior centrosome; Fig. 72 shows what apparently is the fusion of the chromatoid body with the posterior centrosome; and in Fig. 73 the chromatoid body is absent and the posterior centrosome is far down the axial filament and so small that it can scarcely be detected.

Figs. 74 and 75. Later stages of the developing spermatozoön showing the chromatoid body in the cytoplasm at the posterior end. Fig. 74 shows the posterior centrosome still on the filament, while Fig. 75 shows that it had been sloughed off.



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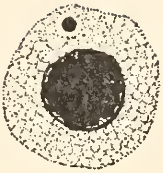

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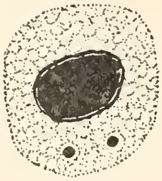

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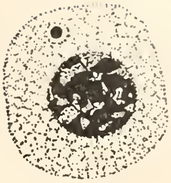

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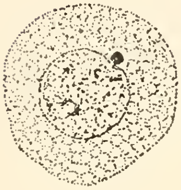

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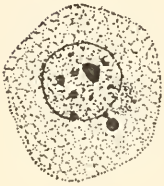

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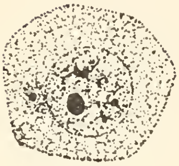

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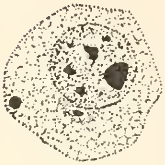

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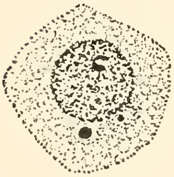

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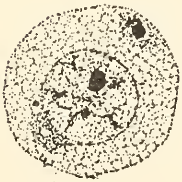

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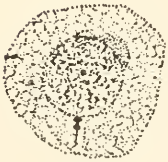

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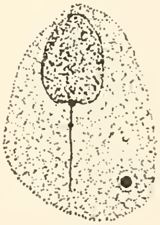

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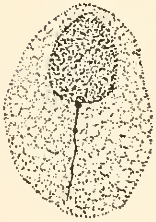

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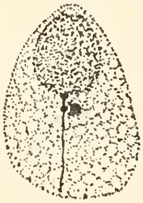

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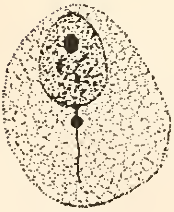

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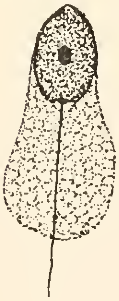

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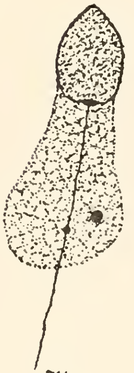

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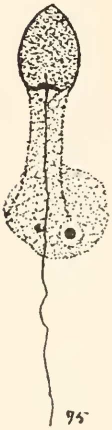

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PLATE VI.

FIGS. 76–79. Later stages of the developing spermatozoön. Fig. 76 shows the chromatoid body far down on the axial filament; Fig. 77 shows the chromatoid body very close to the posterior centrosome; Fig. 78 shows the sloughed-off centrosome, but the chromatoid body is absent; and in Fig. 79 both of these bodies are lacking

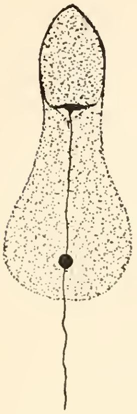
FIGS. 80–83. Cast-off balls of cytoplasm. Fig. 80 shows a small body which apparently is the sloughed-off centrosome; Fig. 81 shows neither the centrosome nor the chromatoid body; Fig. 82 shows both bodies; and Fig. 83 represents the ball of cytoplasm in the process of degeneration.

FIG. 84. A deeply staining body occasionally found in the lumen of the tubule and probably the same thing as the chromatoid body.

FIGS. 85 AND 86. Final stages in the developing spermatozoön. Fig. 85 shows that the cytoplasmic ball is about to be thrown off; and Fig. 86 shows the cytoplasmic mass together with the chromatoid body completely separated from the spermatozoön.

FIG. 87. Side view of a mature spermatozoön.

FIG. 88. A mature spermatozoön.



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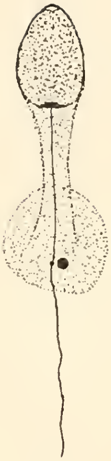

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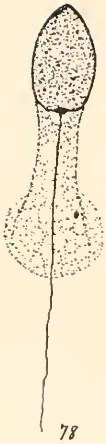

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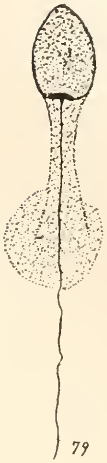

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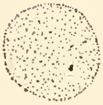

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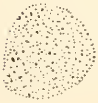

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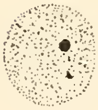

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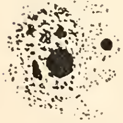

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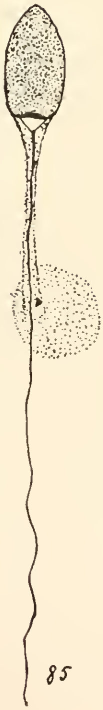

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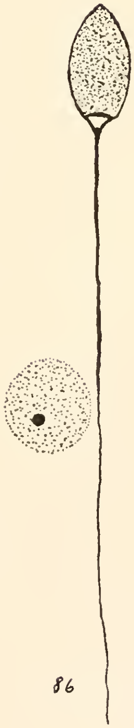

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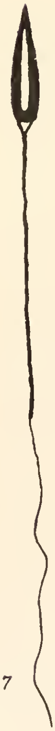

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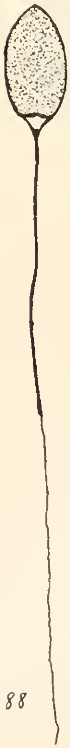

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J. E. WODSEDALEK.

AN EXPERIMENTAL STUDY OF THE AUDITORY POWERS OF THE GIANT SILKWORM MOTHS (SATURNIIDÆ)

C. H. TURNER,

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This is a companion paper to the "Auditory Powers of the Catocala Moths" by C. H. Turner and Ernst Schwarz. The latter paper embodies the results of a field study and this epitomizes a laboratory investigation. The habits of resting quietly upon a tree trunk and of flying, when disturbed, to a nearby tree renders the Catocalæ excellent material for field study; the fasting habits of the Saturniidæ render them equally good material for laboratory work. The paper on the Catocala moths contains both a historical resume and a bibliography; hence they are not needed in this contribution.

In these experiments the following moths were used: 79 specimens of *Samia cecropia* Linn., 104 of *Philosamia cynthia* Drury, 41 of *Callosamia promethea* Drury and 81 of *Telea polyphemus* Cramer. These insects were confined beneath wire dish covers. Each moth was numbered and one insect, in case of mated individuals one pair, was placed beneath a cover.

These experiments were conducted in an out-of-doors insectary the north wall of which is constructed almost entirely out of wire netting. The other three wooden walls are window-less and lined with shelves. These walls and the shelf-rests are supported by the ground. The wooden floor rests on the ground, but is not attached either to the walls or the shelf-rests; indeed, a space of from one to three feet separates the floor from the walls. Suspended from the ceiling by picture wire, there is a heavy swinging shelf. The subjects of these experiments were kept on these shelves. Since I always stood on the floor when sounding any of the instruments, it was impossible for the vibrations to reach the moths by any medium other than the air.

These experiments were conducted in the mornings between five and half past seven and in the afternoons between three and seven. On Saturdays and Sundays experiments were sometimes conducted all day long.

For producing stimuli the following instruments were used: an adjustible organ pipe, with a range for all notes of two octaves and for one note of three; an adjustable pitch pipe, and an Edelmann's Galton whistle. Such moths as responded did so by moving the wings as though about to fly. In the early experiments, before I had many moths on hand, each moth was tested with all of these instruments; because I hoped to determine the upper and lower threshold of hearing for each specimen. Later on, partly because I became convinced that there are theoretical

Table I.

Number: 3–VI–14: 1. *Specimen: Callosamia promethea*, female.

Place: Confined, under a wire dish cover, on the swinging shelf.

Method: At each trial the instrument was sounded five times at intervals of a minute and records made of the moth's behavior.

Trials.	Date.	Time.	Stimulus.	Vibrations Per Second.	Temperature.	Tests.					Remarks.
						1	2	3	4	5	
1	3–VI	6:30	P.P.	680	71	*	*	*	*	*	Response vigorous.
2	4–VI	6:00	P.P.	680	78	*	*	*	*	*	Response vigorous.
3	4–VI	6:10	G.W.	3,480	78	—	—	—	—	—	
4	4–VI	6:15	O.P.	512	78	*	*	*	*	*	Response vigorous.
5	4–VI	6:20	O.P.	256	78	*	*	*	*	*	Response slight.
6	4–VI	6:25	O.P.	128	78	*	*	*	*	*	
7	4–VI	6:30	O.P.	64	78	*	*	*	*	*	
8	6–VI	10:05	P.P.	680	86	—	—	—	—	—	Whistle held in rear.
9	6–VI	10:10	O.P.	512	86	—	—	—	—	—	Whistle held in rear.
10	6–VI	10:15	O.P.	256	86	—	—	—	—	—	Whistle held in rear.
11	6–VI	10:20	O.P.	128	86	—	—	—	—	—	Whistle held in rear.
12	6–VI	10:25	O.P.	64	86	—	—	—	—	—	Whistle held in rear.
13	6–VI	10:30	P.P.	680	86	*	—	—	—	—	Whistle held in front.
14	6–VI	10:35	O.P.	256	86	*	*	*	*	*	Whistle held in front.
15	6–VI	15:00	P.P.	680	96	*	*	*	—	—	Whistle held in front.
16	6–VI	15:10	O.P.	256	96	—	*	—	*	*	Whistle held in rear.
17	6–VI	15:20	O.P.	64	96	—	—	—	—	—	
18	6–VI	15:30	O.P.	256	96	*	*	*	*	*	

Explanation of abbreviations; G.W. means Galton whistle; O.P., organ pipe; P.P., pitch pipe; in the second column, the roman numerals stand for months and the Arabic for days; in the third column, the hours are numbered from 1 to 24, beginning at 1 A.M.

reasons why the thresholds cannot be accurately determined by this method and partly on account of practical difficulties, I confined my experiments to a few notes of the middle range. When I remind you that I often had on hand from fifty to seventy-five moths, you will readily see that it was impossible to test each moth, each time, with the entire range of pitches.

The results of these investigations were recorded upon blanks that were prepared especially for this work. A portion of one of those blanks is reproduced in the preceding table.

After the work on all of the moths had been completed, the contents of these blanks were condensed into the following tables.

TABLE II.

REACTIONS OF GIANT SILK-WORM MOTHS TO SOUNDS.

Name of the Specimen.	Number of Individ.	Number of Trials.	Per Cent. of Responses.										
			0	1 to 9.	10 to 19.	20 to 29.	30 to 39.	40 to 49.	50 to 59.	60 to 69.	70 to 79.	80 to 89.	90 to 100.
Samia cecropia													
Males	38	380	1	0	0	0	1	0	1	2	0	0	33
Females	41	615	0	0	0	1	0	1	5	0	1	1	32
Total	79	995	1	0	0	1	1	1	6	2	1	1	65
Philosamia cynthia													
Males	50	950	19	0	0	2	4	4	9	3	1	1	7
Females	54	875	10	0	1	3	0	1	11	4	4	1	19
Total	104	1,825	29	0	1	5	4	5	20	7	5	2	26
Callosamia promethea													
Males	23	380	4	0	0	0	3	0	5	0	0	1	10
Females	18	495	1	0	0	0	1	0	1	2	5	2	6
Total	41	875	5	0	0	0	4	0	6	2	5	3	16
Telea polyphemus[1]													
Males	39	950	36	0	0	1	0	0	0	0	0	0	2
Females	39	950	39	0	0	0	0	0	0	0	0	0	0
Total	78	1,900	75	0	0	1	0	0	0	0	0	0	2

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[1] The above table does not record the three specimens of *T. polyphemus*, which were used in the special tests recorded on pages 333–334.

TABLE III.

RESPONSES OF *Samia cecropia* TO SOUND.

Instrument.	Pitch Vibra. per Second.	Individuals Participating.			Number of Trials.			Per Cent. of Response.		
		Males.	Females.	Total.	Males.	Females.	Total.	Males.	Females.	Total.
O.P.	64	1	4	5	5	35	40	100	100	100
O.P.	128	2	1	3	10	5	15	50	100	67
O.P.	256	6	13	19	25	75	100	100	100	100
O.P.	512	1	2	3	5	15	20	100	100	100
P.P.	680	26	34	60	310	360	670	94	89	91
P.P.	870	2	2	4	15	10	25	33	0	20
G.W.	3,480	11	19	30	60	100	160	100	70	81
G.W.	4,645	0	1	1	0	5	5		100	100
G.W.	6,200	1	0	1	10	0	10	50		50
G.W.	6,960	0	1	1	0	5	5		100	100
G.W.	9,290	1	0	1	5	0	5	100		100

Explanation of abbreviations used in above table: O.P., organ pipe; P.P., pitch pipe; G.W., Galton whistle (Edlemann's).

TABLE IV.

EFFECT OF AGE ON THE RESPONSES OF *S. cecropia* TO SOUND.

Age in Days.	Individuals Participating.			Number of Trials.			Per Cent. of Responses.		
	Males.	Females.	Total.	Males.	Females.	Total.	Males.	Females.	Total.
0–1	26	23	49	205	200	405	80	80	80
1–2	7	10	17	60	55	115	100	82	91
2–3	5	11	16	30	65	95	100	54	72
3–4	2	12	17	10	120	130	100	100	100
4–5	4	4	8	20	25	45	100	100	100
5–6	2	3	5	10	20	30	100	75	83
6–7	4	3	7	25	30	55	100	83	91
7–8	1	2	3	15	20	35	100	100	100
8–9	1	2	3	10	15	25	100	100	100
9–10	2	4	6	25	30	55	100	100	100
10–11	0	3	3	0	15	15		100	100

TABLE V.

EFFECT OF TEMPERATURE ON THE RESPONSES OF *S. cecropia* TO SOUNDS.

Temperature in F. Degrees.	Individuals Participating.			Number of Trials.			Per Cent. of Responses.		
	Males.	Females.	Total.	Males.	Females.	Total.	Males.	Females.	Total.
50–59	11	6	17	130	70	200	83	53	70
60–69	9	14	23	130	110	240	74	87	80
70–79	4	16	20	40	170	210	100	94	95
80–89	18	27	45	155	235	290	97	96	96
90–99	0	4	4	0	35	35		100	100

TABLE VI.

EFFECT OF MATING ON THE RESPONSES OF *S. cecropia* TO SOUND.

	Number of Individuals.	Number of Trials.	Per Cent. of Responses.
Males:			
Unmated......	31	320	88
Mated	7	120	97
Total.......	38	440	90
Females:			
Unmated......	36	520	86
Mated........	5	55	73
Total.......	41	575	85
Grand total......	79	1,015	88

TABLE VII.

RESPONSES OF *Philosamia cynthia* TO SOUND.

Temperature in F. Degrees.	Individuals Participating.			Number of Trials.			Per Cent. of Responses.		
	Males.	Females.	Total.	Males.	Females.	Total.	Males.	Females.	Total.
60– 69	25	23	48	145	120	265	31	42	33
70– 79	43	43	86	410	420	830	36	60	48
80– 89	23	34	57	245	300	545	33	67	51
90– 99	6	11	17	130	65	195	58	77	67
100–109	0	8	8	0	40	40		88	88

TABLE VIII.

EFFECTS OF MATING ON THE RESPONSES OF *Philosamia cynthia* TO SOUND.

	Number of Individuals.	Number of Trials.	Per Cent. of Responses.
Males:			
Unmated......	47	880	38
Mated........	8	65	31
Total.......	55	945	36
Females:			
Unmated......	50	835	63
Mated........	8	90	56
Total.......	58	925	61

TABLE IX.

EFFECT OF AGE ON THE RESPONSES OF *Philosamia cynthia* TO SOUND.

Age in Days.	Individuals Participating.			Number of Trials.			Per Cent. of Responses.		
	Males.	Females.	Total.	Males.	Females.	Total.	Males.	Females.	Total.
0–1	45	51	96	645	400	1,045	34	58	43
1–2	26	19	45	290	160	450	49	68	57
2–3	12	18	30	160	365	525	38	73	53
3–4	19	19	38	145	105	250	32	58	42
4–5	6	12	18	45	70	115	32	64	48
5–6	1	6	7	5	30	35	100	83	85
6–7	1	1	2	5	5	10	0	100	50
7–8	2	4	6	10	20	30	0	50	34
9–10	0	2	2	0	10	10	0	50	50

TABLE X.

EFFECT OF TEMPERATURE ON THE RESPONSES OF *Callosamia promethea* TO SOUND.

Temperature in F. Degrees.	Individuals Participating.			Number of Trials.			Per Cent. of Responses.		
	Males.	Females.	Total.	Males.	Females.	Total.	Males	Females.	Total.
50–59	1	0	1	5	0	5	100		100
60–69	7	3	10	50	20	70	70	75	71
70–79	10	13	23	125	125	250	72	80	76
80–89	16	18	34	135	250	385	56	76	69
90–99	10	18	28	70	150	220	71	87	82
100–109	0	8	8	0	40	40		63	63

TABLE XI.

EFFECT OF MATING ON THE RESPONSES OF *Callosamia promethea* TO SOUND.

	Number of Individuals.	Number of Trials.	Per Cent. of Responses.
Males:			
Unmated......	19	375	63
Mated........	3	55	82
Total.......	21	430	67
Females:			
Unmated......	15	515	80
Mated........	3	62	80
Total.......	18	575	80

A careful perusal of the tables I–XII. shows that *S. cecropia*, *P. cynthia* and *C. promethea*, respond to a long range of sound waves. Since precautions were taken to prevent vibrations reaching them through any medium other than air, it seems

Table XII.

Effect of Age on the Responses of *Callosamia promethea* to Sound.

Age in Days.	Individuals Participating.			Number of Trials.			Per Cent. of Responses		
	Males.	Females.	Total	Males.	Females.	Total.	Males.	Females.	Total.
0–1	17	13	30	155	105	260	74	76	75
1–0	14	14	28	125	135	260	68	89	79
2–3	11	13	24	60	125	185	50	92	78
3–4	7	10	17	35	75	110	30	75	58
4–5	2	8	10	10	105	115	100	57	61
5–6	1	6	7	5	45	50	0	56	50
6–7	0	1	1	0	5	5		100	100
7–8	0	1	1	0	5	5		100	100

reasonable to conclude that they hear. How about *Telea polyphemus?* Of the seventy-eight individuals whose behavior is recorded in Table II. only three made any responses whatever. Of these three, two gave over ninety per cent. of responses and the other less than thirty. Shall we conclude that *Telea polyphemus* is deaf and that these few responses were due to some factor overlooked by the investigator; or, shall we consider the responses made by all of these moths as expressions of emotion, and attribute the non-responsiveness of *polyphemus* to a sluggish temperament?

To one who has worked much with *Telea polyphemus*, this last suggestion is fascinating; for this moth is exceptionally unresponsive to all ordinary stimuli. The opposite sex is about the only thing that arouses much activity. There is another possibility. *Telea polyphemus* is not a very conspicuous object; indeed, in certain situations, it might be considered protectively colored. It may be that correlated with this inconspicuous coloration is an instinct to remain rigidly immobile in the presence of all ordinary stimuli. To test the matter the following experiments were conducted.

A freshly emerged *Telea polyphemus*, the wings of which had become thoroughly dry, was tested with an organ pipe set to produce 256 vibrations per second. As was to be expected, there was no visible response. The organ pipe was then sounded five times in rapid succession. Immediately thereafter, the insect was roughly handled for a few minutes. It was tossed about, gently squeezed and thrown upon its back. This was repeated over and over again, sometimes in one order and sometimes in another. After the moth had quieted down, the pipe was sounded five times in rapid succession. Each time the pipe was sounded, the moth waved its wings vigorously. At intervals of two hours, this experiment was repeated from early morning until dark. Invariably the moth responded in the same manner. On the following day the experiment was continued with the same moth. The result was always the same.

About a week later, similar experiments were conducted with two other specimens of the same moth. These, like the one used above, were females. With two exceptions, the results were identical. The exceptions were as follows: (1) one of the moths instead of moving its wings vigorously moved them slowly; the other two moths moved their wings so vigorously that they were lifted off of the support; in this case the body remained on the support, although the wings moved each time the whistle blew; (2) on two occasions a moth that had been experimented upon several times, instead of waiting for the five tones that were produced after the handling, waved its wings vigorously to each of the five preliminary notes. Evidently *Telea polyphemus* can hear. These experiments induced in those moths a state of nervous excitability which caused them to respond to the sounds produced.

CONCLUSIONS.

1. It seems certain that all four of the species of giant silkworm moths investigated can hear. Three of the species respond readily to a large range of sounds. The third, *Telea polyphemus*, normally does not respond to sounds; unless remaining as immobile as possible be considered a response. By experimentally causing the moth to associate some disagreeable experience with certain sounds, it can be induced to respond to those sounds.

2. There is much evidence that the responses of moths to stimuli are expressions of emotion. The fact that an insect does not respond to a sound is no sign that it does not hear it. The response depends upon whether or no the sound has a life significance.

A PRELIMINARY ACCOUNT OF SOME CYTOLOGICAL CHANGES ACCOMPANYING DESICCATION.

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The ability of certain rotifers, tardigrades and nematode worms to withstand periods of desiccation has been a subject of investigation for many biologists throughout a period of more than two hundred years. Beginning with von Leeuwenhoek in 1701 and extending to the present time, researches have been carried on at intervals, in the case of the Bdelloid rotifers, with the object of determining whether or not these animals can undergo a true desiccation. The results of the several authors have shown a striking variance, in fact in some cases the conclusions of one worker have been directly opposed to those of some other who used the same species of animal in his experiments. The latest publication upon this subject is that of Jacobs ('09) who worked on the Bdelloid rotifer, *Philodina roseola.* He concludes, after prolonged experimentation and as a result of chemical and physical tests as well as by other indirect methods, that this animal undergoes a true desiccation; that at all times the cuticle is freely permeable to water and gases and that no evidence of a waterproof cyst can be found. He notes further that desiccation is usually followed by a period of reproductive activity. The foregoing conclusions together with others not bearing directly upon the subject of this paper have been confirmed during the course of this study.

Jacobs, while conducting his investigation in a very thorough manner, made no attempt to determine, from a histological or cytological point of view, the condition of the tissues of the desiccated animals as compared with those in the normal individual. To the knowledge of the author no comparison of this sort has been attempted by any investigator up to the present time. At the suggestion of Dr. E. G. Conklin, I have undertaken an inquiry into this last question and present here a brief account of the results obtained to date. A more detailed account based upon further work will appear in a future publication.

The anatomy of the Philodinidæ has been described by Janson (1893). The most conspicuous organs in the living animal are those of the reproductive and the alimentary systems. The latter begins with the buccal cavity at the base of the trochal organs. This narrows down to a short tube leading to the mastax. The mastax, with the digestive glands surrounding it, is followed by the thin-walled esophagus which leads into the thick-walled stomach. The posterior end of the latter is continued by the "*blasendarm*" which leads to the cloaca and thence to the anus. The reproductive organs consist of two more or less spindle-shaped bodies lying on either side of the stomach. These are the ovaries and the vitellaria and they may at all times be recognized by their prominent nuclei. The nephridia with their flame cells are easily observed in the living animal. In the foot are found the glands which secrete a substance which enables the animal to adhere to different objects. The head region contains several large coronal cells with large nuclei.

A cross section of a normal extended *P. roseola* through the mid-body region is shown in Fig. 1. The vitellaria have enormous nuclei, consisting of a central karyosome surrounded by a clear homogeneous area and peripherally by a distinct nuclear membrane. This is the "nucleolar nucleus" of Carnoy and this type of nucleus is characteristic of the greater part of the cells of the animal. The cytoplasm of the vitellarium, which is syncytial in nature, is made up of granules of varying sizes and these granules appear in different patterns particularly when a variety of fixatives are used; it has quite an affinity for nuclear stains as is usual with yolk structures. The cytoplasm of the ovary does not differ essentially from that of the vitellarium. Ovary and vitellarium are each surrounded by a thin membrane. Figs. 1 and 4 show characteristic sections through normal reproductive glands.

The cytological structure of the stomach of the Philodinidæ has been described by Zelinka ('86) in the case of *Callidina symbiotica*, by Janson ('93), and more recently by de Beauchamp ('09), in the case of *Callidina socialis*. The lumen, the position of which in the stomach tissue is not constant, is lined with a heavy ciliated cuticula. Just beneath the cuticula are found longitudinal muscle fibers arranged at regular intervals. The part of the stomach outside the thin layer of muscle fibers is syncitial in nature. With the iron-hæmatoxylin-eosin-lichtgrün stain of de Beauchamp three elements may be distinguished, (1) nuclei having, in general, the same structure as those described for the vitellarium; (2) densely staining granules of great size, not surrounded by a clear area or membrane. These are probably aggregations of food material. (3) Vacuoles of greater or less size which stain with lichtgrün. These last are probably globules of excretory material as de Beauchamp has pointed out. In the latter's description of the stomach of *Callidina socialis* he says that the stomach is not surrounded by an "individualized membrane" but only by a layer of protoplasm which projects at the periphery. In *P. roseola*, as far as I have been able to determine, a true membrane is present (Fig. 1).

The skin of *P. roseola* has practically the same structure as that of other rotifers. It consists of two layers, cuticula and hypodermis. The former is the more densely staining layer and is composed of fine granules closely packed together; the latter is a finely reticulated plasma layer in which cell boundaries cannot be distinguished and in which nuclei are found scattered at irregular intervals. The skin is pliable and may be readily folded at any point. It is difficult to obtain sections in which one or more of these folds do not appear.

The brain of *P. roseola* is of an elongated triangular shape and lies in front of and slightly above the mastax. Zelinka ('88), in the case of *Discopus synaptæ*, has figured the brain as a syncitium in which the nuclei are closely packed together about the periphery, while in the central part is found the "*punkstubstanz*," a finely granular portion without nuclei. In the greater number of cases I have been able to distinguish definite cell boundaries in the case of the cells forming the peripheral layer of the brain of *P. roseola*. The nuclei of these cells are uniformly circular in section and contain a small amount of chromatin scattered in irregular masses through a homogeneous nuclear plasm. The cytoplasm is homogeneous and has the appearance of a colorless fluid. The "*punktsubstanz*" lies approximately at the center of the organ and is granular in structure.

In the fully extended living *Philodina* the ciliated or trochal discs are prominent at the anterior end of the body. The cilia upon these discs by their successive beating give the effect of a revolving wheel. When the animal is disturbed the discs are folded and retracted into the pharyngeal region where they may be observed as oval patches. The alimentary canal is also ciliated throughout almost its entire length.

Of the glandular structures, other than those employed for reproduction, the slime glands of the foot are perhaps most easily seen. These consist of rows of cells whose cytoplasm is alveolar or finely reticular. The nuclei are large and may sometimes be seen in the living animal. The digestive glands in the region of the mastax are similar to the foot glands in structure and staining qualities.

The changes in cell organization which accompany the process of desiccation are fairly uniform in result for all the tissues. Although slight variations have been observed, these are differences of degree and not of kind. Since the cellular elements are larger and hence more easy to observe in the vitellarium, this organ will be considered first.

In a section of the vitellarium of a desiccated *P. roseola* the most noticeable difference from the conditions which are present in the normal tissues are seen in the nucleus. Normally, as was stated before, the nuclear membrane, though definite, is not very thick. Just within the membrane is a ring of homogeneous ground substance or nuclear sap. In the center of the nucleus is found the large, densely staining karyosome. In the dried animal these conditions are exactly reversed. The karyosome may disappear entirely but if this extreme condition does not come about, the structure which remains in the position of the karyosome is similar neither in shape nor in staining qualities to the original element. In extreme cases the central area of the nucleus in the dried organ has exactly the same appearance as the clear area of the normal nucleus. The nuclear membrane becomes heavy and has the appearance of a thick ring (Fig. 5). In most cases it appears to be composed of fine granules closely packed together. Under conditions mentioned hereafter this granular appearance may give place to a dense homogeneous black ring (iron-hæmatoxylin preparations) staining exactly like the normal karyosome. The changes in the cytoplasm, though distinct, are much less marked than the nuclear changes. With the withdrawal of water the cytoplasm increases in density and loses the regular arrangement of its particles which is characteristic of the normal vitellarium (Fig. 4). The yolk granules become arranged irregularly or in small closely packed groups as in Fig. 5. The drying process causes a loss of staining power in the tissue.

The shrinkage of the cytoplasmic portion of the tissues is well demonstrated in the case of the hypodermis. Fig. 1 show as section of this layer of the skin as it appears in the animal living under normal conditions. In a section through the dried animal (Fig. 2) it will be noticed that the hypodermal layer has shrunken markedly, approaching its normal thickness only in those places where the nuclei are located. The nuclei apparently do not diminish in size and they cause a protuberance in the dried hypodermis wherever they are found. The nuclear material is redistributed in the same manner as was described for the vitellarium.

This arrangement of the nuclear elements is found in practically all the tissues of the dried animals. A detailed description of the changes in the other organs would be, for the most part, mere repetition.

As was mentioned above, the cilia in *P. roseola* are well developed, both in the head region and in the digestive tract. It would seem that a fiber of such delicate texture as that of a cilium would not long survive the effects of a removal of moisture. Such, however, is not the case. Not only do the trochal cilia escape serious injury by the desiccation process but the same is also true of those in the digestive canal. Fig. 2 shows a section cut through the infolded trochal discs of a dried animal. There is no sign of any fusion or other abnormal condition of these elements. Each cilium preserves its identity apparently as well as would those of an animal living in a natural environment.

The changes in cell structure attending recovery from desiccation are almost the exact opposite of those just described. In cases where the karyosome has entirely disappeared it begins to form again in its proper position a short time after water is added to the dried animals. The thickened nuclear membrane described above shows a greater affinity for stains at this stage and gradually assumes its normal thickness. Cytoplasmic changes are quite noticeable at this time. In the vitellarium (Fig. 6) it is frequently noticed that the material surrounding the nucleus is aggregated into strands or other irregular patterns. This would seem to indicate that the cytoplasm is more freely permeable to water in certain regions than in others and that the stage represented in Fig. 7 shows a step in the gradual redistribution of extranuclear substance attending recovery from desiccation. In the case of the other organs, as before, the process of recovery is very similar. The elements are much smaller in some cases and hence more difficult to observe but the mechanism as well as the result seems to be the same.

It has been suggested to the author that the cytoplasmic and the nuclear changes taking place in dry seeds might be analogous to the ones in the rotifers just described. With this in mind, sections of the embryo of the common Indian corn, *Zea mais*, were cut, (1) at the time the seeds were fully ripened but had not become entirely dried; (2) after the seeds were thoroughly dried; and (3) after the seeds were well germinated. A section of a typical procambium cell from each of these stages is shown herewith. Fig. 14 shows a cell from a germinating embryo. It will be noticed that the cytoplasm contains many spaces filled with cell sap. The nucleus has a ring of chromatic material just within the nuclear membrane. The nucleolus is vacuolated and does not stain in the same manner as the chromatic ring at the periphery of the nucleus. The nucleolus is surrounded by a clear area which probably consists of fluid material. Fig. 15 shows the conditions which exist when the embryo is partially dried. The chromatic ring thickens, diminishing the space between it and the nucleolus. The latter becomes more compact and the vacuoles disappear. An extreme case of drying is shown in Fig. 16. The cytoplasmic granules are closely and regularly packed together. The clear space in the nucleus has disappeared and the substance of the nucleolus has apparently diffused throughout the nuclear area.

The changes described for the drying corn cells in the last paragraph are at first sight remarkably like those occurring in the rotifer during desiccation. In both rotifer and corn the nucleus contains a nucleolus surrounded by a clear space, while around the two is a chromatic membrane of varying thickness. When water is removed the clear space around the nucleolus disappears and comes into existence again only upon the addition of water. The substance of the nucleolus in both cases diffuses toward the periphery of the nucleus leaving a more or less clear space in the center of the same. In the cytoplasm also there is a parallel between the behavior of the cells of the two forms. Loss of water is attended by shrinkage and a consequent increase in density. The cytoplasmic materials tend to gather in small lumps which remain closely packed together until moisture is again applied.

Whether the seemingly similar processes in these representatives of the plant and animal kingdom are indeed analogous can be determined only after further study.

LITERATURE CITED.

de Beauchamp, P. M.

'09 Recherches sur les Rotiféres: les formations tegumentaires et l'appareil digestif. Arch. Zool. Exp., Paris, Ser. 4, Vol. 10.

Jacobs, M. H.

'09 The Effects of Desiccation on the Rotifer Philodina roseola. Jour. Exp. Zool., Vol. 6.

Janson, F. O. F.

'93 Versuch einer Uebersicht über die Rotatorien-Familie der Philodinaeen. Abh. der Nat. Ver. zu Bremen, Band XII.

Zelinka, C.

'86 Studien über Raderthiere. Ueber die Symbiose und Anatomie von Rotatoria aus dem Genus Callidina. Zeit. Wiss. Zool, Band. 44.

DESCRIPTION OF PLATE I.

FIG. 1. Cross section through the mid-body region of a normal expanded *Philodina roseola*. Leitz compensating ocular 4, obj. 2mm.

Fig. 2. Section of a rotifer kept for eighteen days previous to fixation in an evacuated calcium chloride desiccator. Leitz oc. 4, obj. 2mm.

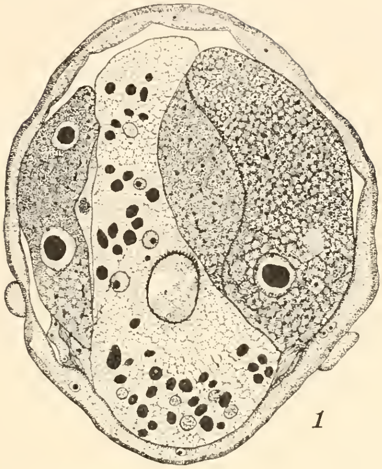
FIG. 3. Section through a normal animal, not expanded. Leitz oc. 4, obj. 2mm.

FIG. 4. Section through the vitellarium of a normal animal. Leitz oc. 12, obj. 2mm.

FIG. 5. Section of the vitellarium of an animal dried in an evacuated desiccator for fourteen days previous to fixation. Leitz oc. 8, obj. 2mm.

FIG. 6. Longitudinal section of an animal recovering from desiccation. The rotifer was kept in an evacuated desiccator for fifteen days, then placed in water for an hour and fifteen minutes, at the end of which time it was fixed. Leitz oc. 8, obj. 2mm.

FIG. 7. Cross section of vitellarium of animal recovering from desiccation. Animal was kept in an evacuated desiccator for six days, then placed in water for one hour, at the end of which time it was fixed. Leitz oc. 8, obj. 2mm.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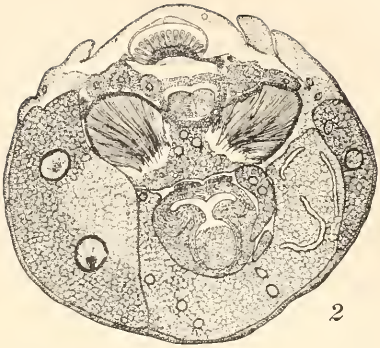
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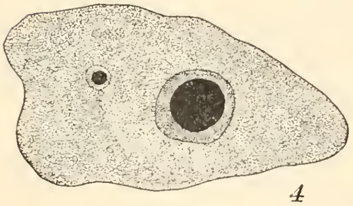
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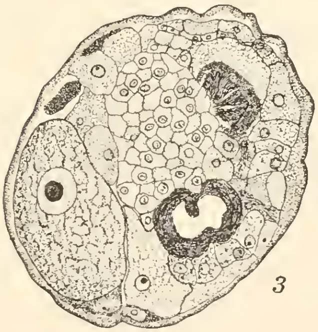
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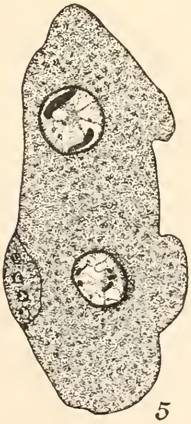
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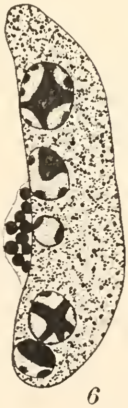
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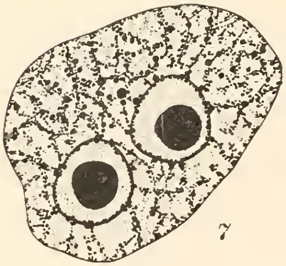
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PLATE II.

FIG. 8. Section of brain of *P. roseola*. Normal active animal. Leitz oc. 8, obj. 2mm.

FIG. 9. Section of brain of a rotifer which was kept in an evacuated desiccator for fourteen days previous to the time of fixation. Leitz oc. 8, obj. 2mm.

FIG. 10. Section of brain of a rotifer which was dried twenty-four hours, put in water for one hour and then killed. Leitz oc. 8, obj. 2mm.

FIG. 11. Section of foot gland cells from a normal active animal. Leitz oc. 12, obj. 2 mm.

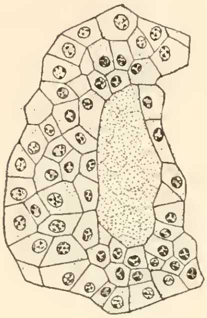
FIG. 12. Section of foot gland ells of a rotifer which was kept in an evacuated desiccator for fourteen days previous to the time of fixation. Leitz oc. 12, obj. 2mm.

FIG. 13. Section of foot gland cells from an animal kept fourteen days in an evacuated desiccator and then placed in water for one and one fourth hours previous to fixation. Leitz oc. 12, obj. 2 mm.

FIG. 14. Section of procambium cell from a germinating corn embryo. Leitz oc. 8, obj. 2mm.

FIG. 15. Section of procambium cell from a partially dried corn embryo. Leitz oc. 8, obj. 2mm.

FIG. 16. Section of procambium cell from a corn embryo dried for a month at room temperature. Leitz oc. 8, obj. 2 mm.



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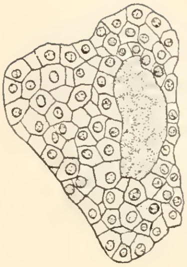

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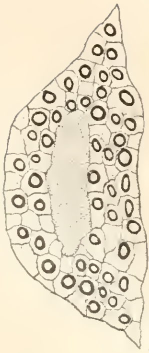

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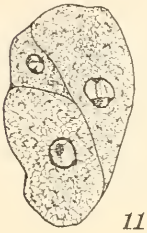

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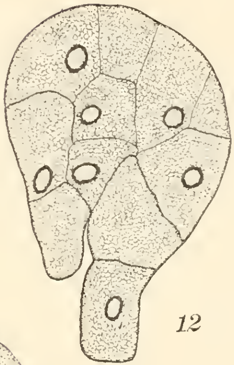

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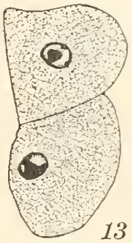

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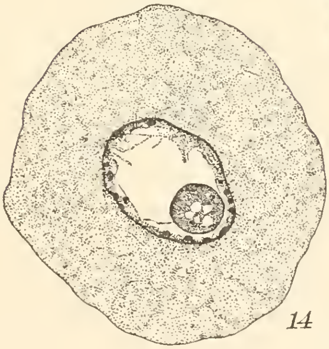

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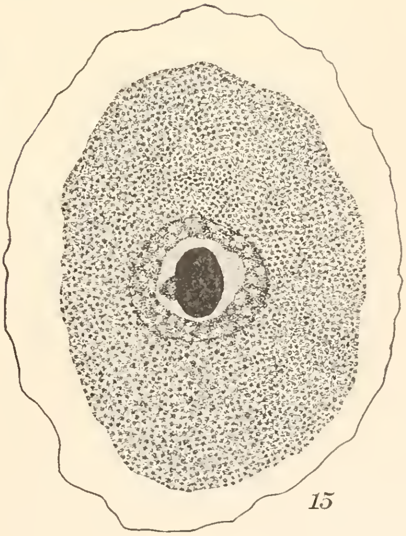

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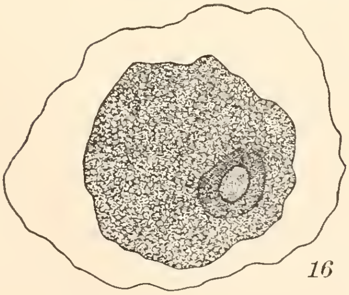

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LOUIS M. HICKERNELL.

REGULATION IN VORTICELLA.

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(From the Department of Biology, Reed College, Portland, Oregon.)

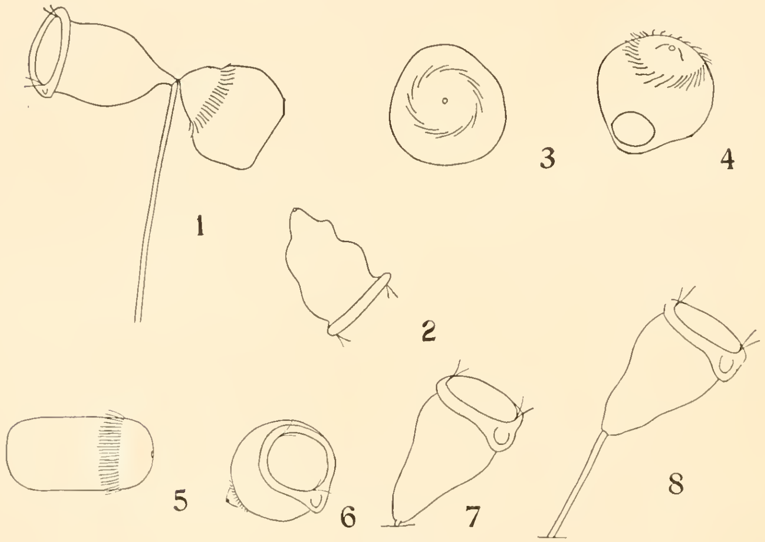
It is a fact well known to students of regeneration that one part of an organism may exert a measurable influence over the growth and development of another. This has been demonstrated for many of the Metazoa under varying forms. The removal of the head of a planarian liberates, as it were, the postjacent tissue, out of which a new head is fashioned. Among macruran crustaceans, the loss of the larger chela of an asymmetrical pair has been shown many times to be succeeded by an accelerated growth of the smaller chela and a subsequent retardation in the regeneration of the lost chela so that, in the presence of the small chela grown large, it remains the smaller of the two. Finally—not to multiply instances needlessly—when a short length of the column, with hydranth, is cut away from the hydroid *Corymorpha*, no development beyond closure of the wound occurs proximally until the hydranth is removed from the distal end. In this respect, the behavior of *Corymorpha* may be contrasted with the behavior of the planarian, since in the latter the presence of the original head on the anterior piece does not inhibit the development of a tail posteriorly. The hydranth in *Corymorpha* appears somehow to inhibit, in short pieces, even the development normally to be expected at the aboral end.

Among the Protozoa, instances of this last sort seem so generally to have escaped record, that we have thought it desirable to describe a similar domination in *Vorticella* sp. of one part over another.

When *Vorticella* divides, the fission plane passes approximately through the center of the organism from oral disk to a point immediately to one side of the contractile stalk. Of the zoöids thus formed, one remains attached to the original stalk, while the other swims away by means of cilia which, during the last phases of the process of fission, have appeared in a circlet near the aboral pole (Fig. 1).[1]

Why do such cilia not appear on the stalked zoöid?

It may be noticed in the last stages of fission that the zoöid destined to become free retains its connection by a slender protoplasmic strand with the body of the stalked zoöid, not directly with the stalk itself. This fact suggests what has proven to be the correct view, namely, that cilia which would normally develop on every individual are able to show themselves only when sufficiently isolated physiologically from the stalk. Such isolation exists when the connection between the separating zoöids is reduced to a narrow strand.



Figs. 1–8.

This view was reinforced by the familiar fact that, upon becoming attached to the substrate, the free zoöid gradually loses its cilia as its stalk develops. In the normal life history, then, aboral cilia develop in isolation from the stalk and disappear with the development of the stalk.

The test was applied by cutting a stalked zoöid quite away from the stalk. This was accomplished under a binocular, by means of a sharp dissecting needle. A typical case is shown in Figs. 2–8. Soon after the cutting, the zoöid (Fig. 2) settled down on its oral surface. In an hour, cilia began to push out in a circlet near the aboral pole. They elongated rapidly, and began to beat around the oral-aboral axis (Fig. 3). The oral disk turned in upon itself in the manner characteristic of the normally free zoöid (Fig. 4). One hundred and five minutes after the operation, the zoöid swam away (Fig. 5), indistinguishable in every respect from the normally free form. After five minutes of active locomotion, it came to rest on its aboral end, became attached, and unfolded its oral disk (Fig. 6). At once the stalk began to grow and the aboral cilia to disappear. In ten minutes no aboral cilia were to be seen (Fig. 7). Two hours and a half later, the organism appeared as in Fig. 8.

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[1] The figures have not been drawn with a camera. Their scale varies somewhat.

The development of the stalk appears to be dependent on contact at the aboral end; while the development of aboral cilia is conditioned by physiological isolation from the stalk whether achieved experimentally or by a narrowing of protoplasmic connection in the ordinary course of fission.

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